Proceedings of the 39th Annual Aquatic Toxicity Workshop: September 30 – October 3, 2012, Sun Peaks, British Columbia

Comptes rendus du 39^{e} atelier annuel sur la toxicologie aquatique: du 30 septembre au 3 octobre 2012, Sun Peaks, Colombie-Britannique

Editors/Éditeurs

J. Harkness , G. van Aggelen , C.J. Kennedy , R. Allen Jarvis and /et L.E. Burridge

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Editors/Éditeurs

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Preface/Préface

The 39th annual Aquatic Toxicity Workshop (ATW) was held at the Delta Sun Peaks in Sun Peaks Municipality, British Columbia, from September 30 to October 3, 2012. The Workshop included two plenary presentations, 54 platform and 29 poster presentations, and a special panel discussion on the future of ATW. Total attendance was 166.

This workshop was one of a continuing series of annual workshops in Canada on aquatic and environmental toxicology, covering topics from basic aquatic toxicology to applications in environmental monitoring, setting of regulations and guidelines, and the development of sediment and water quality criteria. These workshops emphasize an informal exchange of ideas and knowledge on the topics among interested persons from industry, governments and universities. They provide an annual focus on the principles, current problems and approaches in aquatic toxicology. These workshops are administered by a Board of Directors and organized by local organizing committees. The Proceedings are published with the support of the Department of Fisheries and Oceans.

L' 39ième Atelier annuel sur la toxicité aquatique a eu lieu au Delta Sun Peaks à Sun Peaks, Colombie-Britannique, le 30 septembre au 3 octobre, 2012. L'atelier a donné lieu à deux communications lors de séances plénières, 54 présentations orales et 29 présentations par affiche, et une table ronde sur l'avenir de l'Atelier sur la toxicité aquatique. Cent soixante-six personnes ont assisté à l'atelier.

L'atelier a permis de poursuivre les discussions tenues annuellement au Canada sur la toxicologie aquatique et l'écotoxicologie. Ces ateliers annuels organisés par un comité national constitué légalement réunissent des représentants des secteurs industriels, des administrations et des universités que le domaine intéresse. Ces derniers y échangent des idées et des connaissances sur les notions fondamentales de la toxicologie aquatique, mais aussi sur son application pour la surveillance de l'environnement, l'élaboration de lignes directrices et de règlements, et la définition de critère pour les sédiments et pour la qualité de l'eau. Ils passent également en revue les principes de la spécialité, de même que les questions d'actualité et les méthodes adoptées dans le domaine. Les comptes rendus sont publies l'aide du ministre des Pêches et Océans.

Editors' comments/Remarques des éditeurs

This volume contains papers, abstracts or extended abstracts of all presentations at the workshop. An author index is also included. The papers and abstracts were subject to limited review by the editors but were not subjected to full formal or external review. In most cases, the papers are published as presented and therefore are of various lengths and formats. Comments on any aspects of individual contributions should be directed to the authors. Any statements or views presented here are totally those of the speakers and are neither condoned nor rejected by the editors. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

The editors would like to thank Dr. Jill Watson for her assistance in the preparation of these proceedings.

Ces comptes rendus sont publiés en deux volumes, en raison de leur longueur, ils renferment le texte intégral ou le résumé de toutes les communications présentées aux ateliers. Un index des auteurs est aussi inclus. Les communications et les résumés ont été revus sommairement par les éditeurs, mais ils n'ont pas fait l'objet d'une revue exhaustive en bonne et due forme ou d'une revue indépendante. La longueur et la forme des communications varient parce que ces dernières sont pour la plupart publiées intégralement. On est prié de communiquer directement avec les auteurs pour faire des remarques sur les travaux. Toutes les déclarations et opinions paraissant dans le présent rapport sont celles des conférenciers; elles ne sont ni approuvées, ni rejetées par les éditeurs. La mention de marques de commerce ou de produits commercialisés ne constitue ni une approbation, ni une recommandation d'emploi.

Les rédacteurs en chef voudraient remercier Jill Graham dans la préparation de ces comptes rendus.

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The 2012 winner of the MSc thesis award was Eric Franz, University of Saskatchewan.

An *in situ* assessment of selenium bioaccumulation from water, sediment, and dietary exposure pathways using caged *Chironomus dilutus* larvae (PL)

FRANZ, E. [1]***, WIRAMANADEN, C.*** [2]***, JANZ, D.*** [3] ***and LIBER, K.*** [3]

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Elevated selenium (Se) can pose a risk to fish and bird populations in aquatic ecosystems as a result of the trophic transfer of selenium from the base of the aquatic food web. The aim of this study was to document how route of exposure (surface water vs. sediment) affects selenium bioaccumulation in the benthic invertebrate *Chironomus dilutus* at selected sites downstream of a metal mine in northern Canada. An *in situ* approach was used involving caged, laboratory-reared *C. dilutus* larvae exposed to site-specific whole-sediment and surface water, or surface water only, for a period of 10 days at selected sites with a range of differing sediment and selenium characteristics. Results showed low levels of selenium bioaccumulation, across all sites, when *C. dilutus* larvae were exposed to selenium in the surface water at concentrations ranging from less than 1 μg $Se \cdot L^{-1}$ to 12 μg $Se \cdot L^{-1}$. However, whole-body selenium concentrations in larvae exposed to sediment were elevated compared with larvae exposed to surface water across all sites. In response to these findings, a second *in situ* experiment was conducted to investigate the importance of dietary selenium (biofilm and detritus) vs. whole-sediment as an exposure pathway. Larvae exposed to sediment detritus (top 2-3 mm of sediment) from the exposure site had the highest selenium concentrations after 10 d of exposure (15.6 ± 1.9 $\mu g \cdot g^{-1}$) compared to larvae exposed to whole-sediment (12.9 ± 1.7 $\mu g \cdot g^{-1}$) or biofilm (9.9 ± 1.6 $\mu g \cdot g^{-1}$). Biofilm had lower total selenium concentrations than the detritus and whole-sediment fractions, but nearly 80% of the selenium was present as organic selenides similar to selenomethionine. Biofilms appear to be an enriched source of organic selenium and are important food items for many benthic consumers. This research demonstrates the utility of field-based *in situ* approaches for assessing contaminant bioavailability and the dominant exposure pathways in aquatic environments.

The 2012 winner of the BSc Honours thesis award was Danielle Louise Gordon, University of Prince Edward Island.

The effects of the pesticides atrazine, linuron, and vinclozolin on steroidogenesis in the amphibian *Xenopus tropicalis* (PO)

GORDON, D.L. [1]***, VAN DEN HEUVEL, M.*** [1] ***and HOGAN, N.*** [2]

[1]*University of Prince Edward Island,* [2]*University of Saskatchewan*

Endocrine disrupting chemicals can alter the reproductive health of vertebrates, and studies assessing the mechanism of action of these chemicals have focused on their ability to directly bind to steroid hormone receptors. Few tests have been developed to assess functional reproductive responses to these compounds; therefore, the aim of this study was to develop a sensitive *in vitro* bioassay that identifies chemicals with the potential to alter sex hormone production in the amphibian ovary. The second aim was to determine whether the pesticides atrazine, linuron, and vinclozolin are able to disrupt steroidogenesis through changes in expression of steroidogenic genes in the amphibian *Xenopus tropicalis*. An *in vitro* protocol for determining steroid production and measuring the expression of target genes was optimized through a time course experiment with ovarian tissues exposed to the stimulator of steroidogenesis, human chorionic gonadotropin (hCG), for 2, 4, 8, and 16 hours. It was determined that an optimal incubation period to measure both endpoints was 8 hours. The impact of each pesticide on steroidogenesis was evaluated following incubation with 0.1, 1 and 10 $\mu g \cdot mL^{-1}$ for 8 hours. Atrazine caused an increase in testosterone and a decrease in estradiol production. A decrease in testosterone was observed after incubation with linuron and vinclozolin, and linuron also decreased estradiol levels. An increase in StAR mRNA following linuron exposure was the only change in transcript levels. The results demonstrate that the *in vitro* bioassay was able to predict the ability of chemicals to alter steroid biosynthesis, but that changes in hormone production may not be mediated through changes in levels of steroidogenic genes.

Intertidal biodiversity in the Howe Sound region of British Columbia (Canada) and potential for post-industrial habitat recolonization and recovery (PL)

MARLATT, V. [1] ***and BARD, S.*** [2]

[1]*University of the Fraser Valley,* [2]*SNC-Lavalin*

Studies conducted in 1990-1993, 2004 and 2012 showed that biodiversity of intertidal species in the mid- to low-tide zone decreased significantly as exposure to pulp mill effluent increased along pollution gradients in Howe Sound. Diversity was also assessed adjacent to the Brittania Beach before and after successful treatment of mine drainage effluent. During the earliest Howe Sound surveys, two pulp mills (Woodfibre and Port Mellon) were operational, and since the 1990s successful pollution abatement was undertaken to decrease the toxicity of marine effluent. This research describes the recovery of intertidal biodiversity at the most highly impacted sites, Darrel Bay, proximal to the currently decommissioned Woodfibre pulp mill, and Brittania Mine beach. At Darrel Bay, intertidal biodiversity increased from 6 species in 1990s to 12 species in 2004 post-pollution abatement, to 15 species in 2012 post-mill closure. Brittania Beach had zero macroscopic intertidal species during 1990 surveys, post-effluent treatment 4 species were observed in 2004, and in 2012 over a dozen species were present. Experiments investigating whether recolonization and recovery are accelerated at the Darrel Bay site by providing additional rocky intertidal habitat through rock transplantation to the mid-low tide zone are underway. Recruitment of several species to naïve rock was detected by 4 weeks, but mortality of some of these species was observed by 6 weeks. These studies indicate increases in species diversity following pollution reduction; however, further time and, potentially, remediation of historical contamination with habitat augmentation are likely necessary for recolonization to the level of reference sites.

Use of an innovative, controlled, microcosm-approach to distinguish and identify potential causes and effects associated with Kraft pulp mill effluent discharge (PL)

THOMAS, G.P. [1]***, MACKAY, A.*** [1] ***and CALDICOTT, A.*** [1]

[1]*G3 Consulting Ltd.*

Identification and confirmation of cause of any identified effects associated with anthropogenic discharges are often complicated by dynamic and complex receiving systems, confounded by numerous other historic and current anthropogenic discharges

and high system natural variability. A multi-treatment, flow-through microcosm was employed to simulate *in situ* marine conditions along a concentration gradient of effluent discharge. Sediments obtained from the receiving system, which contained associated indigenous benthic invertebrate communities, were collected and exposed to direct pulp mill effluent discharge at varying concentrations as well as a sea water control (0% effluent concentration) for up to 45 days. Following exposure, treatments were compared with baseline and control conditions by examining differences in biophysical sediment endpoints and benthic invertebrate community metrics. This presentation will discuss this novel experimental approach and its application to Environmental Effects Monitoring and other assessments of discharge to marine and freshwater systems.

Risk-based remediation of an intertidal area at the former Versatile Shipyards (PL)

WAUTERS, J. [1] ***and BAILEY, H.*** [2]

[1]*Next Environmental Inc.,* [2]*Nautilus Environmental*

Lot 3 is an area of intertidal sediment at the former Versatile Shipyards in North Vancouver, once the largest shipyard in British Columbia. The shipyards area is progressively being redeveloped into a new waterfront community, and Lot 3 is undergoing remediation to obtain a Certificate of Compliance from the Ministry of Environment to allow occupancy of the new development. Lot 3 was initially characterized by heavy metal contamination as a result of infilling with waste and the presence of an overlying coppersmith shop. Complete excavation of the Site to remediate to numerical standards was cost prohibitive because of the location in the intertidal zone and access restrictions due to an overlying building. A risk-based approach was then chosen to remediate the Site. Porewater was initially characterized as toxic using 48-hour mussel bivalve larval development tests. Consequently, the toxicity of porewater and sediment were further characterized with several species representing a wide range of ecological and physiologically relevant endpoints. These tests included polychaete survival and growth and amphipod survival tests on whole sediment, and bivalve larval development and echinoderm fertilization tests on porewater. Larval mussels were shown to be the most sensitive receptor, and they were retained as the receptor of concern for post-remediation monitoring. Subsequently, to reduce the porewater concentrations to risk-based standards, hazardous waste was excavated. Following remediation, porewater concentrations decreased significantly. The results of subsequent tests with larval mussels suggested that the objective of eliminating the toxicity of porewater prior to entering the receiving environment had been achieved.

A new approach for developing risk-based, direct contact sediment quality guidelines and standards (PL)

BRIGHT, D. [1]

[1]*Hemmera*

Canadian Council of Ministers of the Environment (CCME) sediment quality guidelines and their United States counterparts have been widely criticized, since the derivation protocol implicitly assumes a causal relationship between the substance of interest and an ecotoxicological response for each of a large series of paired sediment chemistry-biotic response data that are obtained from field studies. This is in spite of the fact that the sediment sample of interest almost invariably contains a complex contaminant mixture as well as ammonia and free sulphides. The CCME Interim Sediment Quality Guidelines are further influenced by a high degree of reliance on a narrow set of biotic response types (e.g., based on data for amphipod laboratory toxicity tests). An artefact of the protocol is that the more data, the more over-conservative the calculated effects thresholds. In addition, the basic derivation protocols are not conducive to the development of site-specific, risk-based sediment assessment and remediation objectives (i.e., Tier II objectives). This talk describes an alternative derivation protocol, using field-based/correlational data for sediment chemistry-biotic responses, to construct species sensitivity distributions (SSDs) for extirpations of individual taxa from benthic communities. The approach, while based on the same type of data behind the CCME sediment quality guidelines, can also accommodate more diverse fauna and endpoints. The approach can be applied generically, but is ideally suited for site- and ecosystem-specific risk characterization. Finally, the re-constructed extirpation SSDs allow the sensitivity of standardized laboratory toxicity test organisms to be defined relative to macrofauna in the field, allowing for significant advances in laboratory-field extrapolations and the further evaluation of ecological relevance of effects on individual taxa.

PCB-associated health risks in ringed seals *(Pusa hispida)* from a point source release of PCBs at a military station in Arctic Canada (PL)

BROWN, T. [1]***, ROSS, P.*** [2]***, HELBING, C.*** [3]***, FISK, A.*** [4] ***and REIMER, K.*** [5]

[1]*University of Victoria / Fisheries and Oceans Canada,* [2]*Fisheries and Oceans Canada,* [3]*University of Victoria,* [4]*University of Windsor,* [5]*Royal Military College of Canada*

Despite the widespread contamination of aquatic food-webs by polychlorinated biphenyls (PCBs), few studies have unequivocally demonstrated adverse effects specifically attributed to this industrial chemical on marine mammals. Causal evidence linking PCBs to toxic effects in free-ranging marine mammals is generally confounded by

the highly complex contaminant mixtures to which they are exposed. Elevated levels of PCBs have been measured in ringed seals (*Pusa hispida*) from the north Labrador coast. Although long-range transport of PCBs through air and ocean currents delivers PCBs and related compounds to Labrador, local sources of PCBs from military facilities along the Labrador coast also exist. Saglek, a former 'Pole Vault' military radar station, is one of two sites where PCB releases have contaminated the adjacent marine environment. Approximately 25% of ringed seals sampled from 2009-2011 had levels of PCBs that exceed health effects thresholds for another pinniped species, the harbour seal (*Phoca vitulina*). In order to evaluate the implications for ringed seal health, we developed quantitative real-time PCR assay to evaluate the mRNA expression of contaminant-sensitive endpoints, including transcripts encoding the aryl hydrocarbon, thyroid hormone, estrogen, and retinoic acid receptors. Preliminary results show that higher mRNA expression of the AhR receptor and thyroid receptor are correlated with increasing levels of PCBs. The combination of gene expression endpoints, contaminant profiles, and insight into habitat use and feeding ecology afforded us a unique opportunity to explore source, transport, fate of PCBs in a coastal area, and assess the effects of PCBs on the health of ringed seals. Results are relevant not only in terms of the health of the ringed seal population, but also the local Inuit who rely heavily on seals and fish for their subsistence.

Will small hydro have a quicksilver lining? (PL)

RODGERS, D. [1] ***and HARRIS, R.*** [2]

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Mercury has become a significant concern for large-scale hydroelectric development, as methylmercury (MeHg) concentrations in piscivorous fish have been observed to increase <2X up to 7X following flooding. Concerns have also been expressed with regard to potential increases in fish MeHg for small hydro developments, although data are limited. Our analysis suggests that to minimize the potential for post-impoundment increase of MeHg in fish, the ideal reservoir would be sited on bedrock, with upland forest preferred to wetlands and where possible, flooding should be limited to the annual high water level. The reservoir should have steep contours with limited littoral area and a high flow through rate to prevent stratification. From a mechanistic viewpoint, vegetation removal or burning prior to impoundment would be expected to reduce the post-impoundment elevation of nutrients and organic carbon in the flooded zone and limit the magnitude of the post-impoundment increase in bacterial MeHg production. However, recent studies indicate that both logging and forest fires may increase mercury release in the watershed, at least over the short term, and mesocosm studies have yielded mixed results. The impacts and contribution of these key factors to the potential increase within small hydro reservoirs, and the application and limitation of a simple regression model that predicts peak fish mercury levels on the basis of flooded area, total area, and mean annual flow will be discussed.

Use of an innovative bioassessment technique (Benthic Transplant Devices©; BTD) to distinguish confounding current and historic anthropogenic effects and natural system influences from current, effluent-associated, discharge-related effects (PL)

THOMAS, G.P. [1]***, MACKAY, A.*** [1] ***and CALDICOTT, A.*** [1]

[1]*G3 Consulting Ltd.*

Benthic invertebrates are often used as a tool in environmental monitoring to help determine the effects of industrial discharges on marine and freshwater systems. Distribution and abundance of benthos living in sediments can provide useful comparisons of habitat health between areas exposed to industrial influences and those considered to contain background communities. Experiments involving benthic organisms *in situ* may be affected by confounding influences from historical legacies and system (e.g., sediment) variability, and it is often difficult and expensive to define ambient ecological conditions. This presentation will discuss a bioassessment technique (Benthic Transplant Devices©; BTD) developed by G3 Consulting Ltd. and its successful use as a means to assess subtle industrial discharge effects on sediment and associated biological communities. BTDs were designed to reduce confounding and historical influences, distinguish from natural ambient system variability, and allow for study designs that can involve meaningful spatial and temporal assessment of industrial influences in a complex environment. By relocating indigenous benthos populations and substrate to areas of exposure while maintaining isolation of substrate from confounding effects, BTDs offer a cost-effective way to determine ecological conditions and current industry related effects. Methodologies, design, findings, and advantages and disadvantages will be discussed, as well as an examination of future objectives and potential uses of BTDs for application in environmental monitoring.

Application of laboratory bench scale testing in bioremediation strategies (PL)

HENSON, E. [1]***, OOSTERBROEK, L.*** [1]***, MARQUES, L.*** [1]***, EHMAN, C.*** [1] ***and MCCLURE, T.*** [1]

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Remediation of contaminants occurs naturally by abiotic and biotic mechanisms, but can be accelerated through bioremediation treatments including bioventing, bioaugmentation, and biostimulation to stimulate microbial growth and metabolism. Bioremediation efficacy varies greatly between sites due to changing environmental conditions, soil characteristics, contaminant composition/complexity, and indigenous

microbial communities. Thus, assessment of the feasibility of implementing bioremediation and definition of optimal treatment conditions for specific sites are imperative to ensure efficacy of the process. Laboratory bench-scale testing can be used to determine the optimal treatment conditions for each specific site.

The process involves designing microcosms which mimic and maintain environmental site conditions by controlling temperature, lighting, oxygen requirements etc. and monitoring several environmental factors concurrently and over time. These factors include direct measurements of contaminant levels through analytical techniques, measuring microbial population dynamics (e.g., quantifying hydrocarbon-degrading bacteria, total heterotrophic bacteria, evaluating for specific microbes using PCR and qPCR technologies); and where applicable, measuring bio-availability of contaminants using prescribed soil toxicity tests (e.g., Microtox). Methods and results from several bench-scale bioremediation projects will be presented.

Sediment contamination in the St. Marys River Area of Concern: Research to support informed management decisions (PO)

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[1]Algoma University, [2]Algoma University / Sault Ste. Marie Innovation Centre, [3]Environment Canada, [4]Ontario Ministry of the Environment, [5]Sault Ste. Marie Innovation Centre

The St. Marys River connects Lake Superior and Lake Huron and is often referred to as the "Hub of the Great Lakes." Since the early 1900s, the river has received industrial and municipal wastewater, which has resulted in sediment contamination with petroleum hydrocarbons, polycyclic aromatic hydrocarbons, oils/grease, and metals such as chromium, iron, and zinc. Because of the extensive contamination and other environmental concerns, the St. Marys River was designated as one of 43 Areas of Concern (AOCs) under Annex 2 of the 1987 Canada-US Great Lakes Water Quality Agreement. The agreement requires the development of a Remedial Action Plan (RAP) for AOCs. Under the Canadian portion of the RAP, science-based evidence is being collected in support of the Canada-Ontario Decision-Making Framework for Assessment of Great Lakes Contaminated Sediment. This includes an assessment of sediment toxicity, benthic community structure, pore water chemistry, surficial and at-depth sediment chemistry, modeling of sediment transport and fate (including new sediment deposition), and geotechnical assessment of the sediment profile with depth. Collectively, these studies will provide the data needed to support informed management decisions specific to the complex mixture of contaminants within the St. Marys River AOC.

Federal environmental quality guidelines for PFOs and comparison to levels in fish from Canadian waters (PO)

DIXIT, S. [1]***, ROE, S.*** [1]***, COWELL, S.*** [1]***, KHERA, N.*** [1]***, GEWURTZ, S.*** [1]***, MCGOLDRICK, D.*** [1]***, BACKUS, S.*** [1] ***and SPRY, D.*** [1]

[1]*Environment Canada*

Perfluorooctane sulphonate (PFOS) belongs to a larger group of fluorochemicals called perfluorinated alkyl compounds. PFOS, its salts and its precursors are entering in Canadian environment in a quantity that has, or may have, an immediate or long-term harmful effect on the environment and biological diversity. Development of draft Federal Environmental Quality Guidelines (FEQGs) is described for water, fish tissue, wildlife diet (mammalian and avian), and bird egg. These FEQGs can aid in preventing pollution by providing targets for acceptable environmental quality, can assist in evaluating the significance of concentrations of chemical substances currently found in the environment (monitoring of water, sediment, and biological tissue), and can serve as performance measures of the success of risk management activities. The FEQGs for PFOS developed here are used as a tool to interpret PFOS data collected by the Environmental Monitoring and Surveillance program of Canada's Chemicals Management Plan. For 2007 to 2009, top predator fish were sampled at 21 sites across Canada representing 11 watersheds. PFOS levels varied considerably, with the highest concentrations observed in lake trout from the Great Lakes. To provide a long-term perspective of PFOS in lake trout, archived samples were analyzed from Lake Ontario from 1979 to 2008. In all years and all cases, concentrations were at least 1 order of magnitude below FEQG for fish tissue. In contrast, the PFOS levels could be of concern to fish-eating wildlife since levels in lake trout were higher than the FEQGs for wildlife diet. Because FEQGs are preventive not predictive, wildlife population health assessments would be necessary to determine whether negative impacts are actually occurring.

Sensitivity of white sturgeon *(Acipenser transmontanus)* to selected environmental pollutants (PO)

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Populations of sturgeons (Acipenseridae) are threatened throughout the world and numbers of individuals in North America, Asia, and Northern Europe have been decreasing over the past century. In North America, populations of white sturgeon (WS; *Acipenser transmontanus*) are declining, primarily due to poor annual recruitment, and have been listed as endangered in parts of north-western USA and British Columbia, Canada. Pollution has been hypothesized as one potential cause for poor recruitment in the Columbia, Fraser, and Sacramento-San Joaquin rivers and their tributaries.

Specifically, there are concerns about the potential toxicity to WS early life stages (ELS's), a period of development when fish are considered to be most sensitive to the exposure of contaminants. WS live in close proximity to sediments, and thus may be at particular risk from effects of pollutants associated with sediments. Little is known about the potential toxicity of inorganic and organic pollutants, such as metals and dioxin-like compounds, to WS, or the tolerance of WS in comparison to other fishes. Here, we present the results of studies that investigated effects of selected metals and an arylhydrocarbon receptor (AhR) agonist to ELS WS. WS were exposed to metals both in water and in sediments collected from the upper Columbia River that are hypothesized to be contaminated with metals. In addition, biochemical and molecular effects of the model AhR agonist, β-naphthoflavone (βNF), on juvenile WS were investigated. ELS WS were relatively sensitive to cadmium (Cd), copper (Cu), zinc (Zn), and lead (Pb), but LC values for acute and chronic exposures were greater than the water quality criteria for both the United States and Canada. WS were responsive to AhR agonists and could be among the more sensitive fishes with regard to inducibility of CYP1A. Although the liver is the main location of these responses, other organs such as the gill and intestine have the capacity to respond, as evidenced by both the high level of induction in EROD activity as well as the induction of both CYP1A and AhR transcript abundance. Preliminary results of the sediment exposure study indicate that exposure to contaminants through sediments in the upper Columbia River is unlikely to significantly contribute to the poor recruitment of WS.

Evaluation of the efficacy of successive municipal wastewater treatment levels using transcriptomic endpoints of a bullfrog C-fin assay (PL)

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The abundant use of pharmaceuticals and personal care products (PPCPs) is an emerging concern for municipal wastewater treatment systems. PPCPs are often classified as endocrine disrupting compounds (EDCs). Much of the focus of EDC research to date has been on sex hormones. Thyroid hormone (TH) effects, although more broad-ranging than sex hormones, are often overlooked in ecotoxicology. While TH is crucial in early life stages of mammalian development, the effects of perinatal TH are paralleled and more apparent in amphibians. TH causes metamorphosis of the juvenile tadpole to the adult frog. We have developed a method to rapidly screen for EDCs in complex mixtures such as wastewaters in *Rana catesbeiana* tadpoles using the cultured tail fin (C-fin) assay. The C-fin assay uses eight functionally athyroid, premetamorphic tadpoles. Twelve 4-mm tail fin biopsies are collected from each tadpole and placed into tissue culture wells containing serum-free medium. Each biopsy is then exposed to different treatment conditions of various wastewater concentrations in the presence or absence of TH or estrogen. After 48 hours, the biopsies are collected, the RNA is isolated, and quantitative real time polymerase chain reaction (QPCR) is performed. Transcripts important in hormone- and stress-signaling pathways are used as the first indicators of endocrine perturbations. We are using the C- fin to study the potential biological effects of successive levels of municipal wastewater treatment: influent, primary treated, and secondary treated wastewaters. Results indicate that secondary treatment of wastewater is a critical step in reducing perturbations of hormone and stress signaling pathways.

Investigating the estrogenicity of tertiary sewage treatment plant effluents: An evaluation of the osmoregulatory and endocrine effects on Chinook salmon (PL)

OSACHOFF, H. [1]***, FURTULA, V.*** [2]***, HARKNESS, J.*** [3]***, VAN AGGELEN, G.*** [2] ***and KENNEDY, C.*** [4]

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In British Columbia (BC), limited knowledge exists of the profile or concentration of estrogenic substances in sewage treatment plant (STP) effluents. Thus, chemical and biological evaluations for estrogenicity are important in determining implications to the environment. In a preliminary evaluation, three out of six BC STP effluents were found to contain estrogenic hormones. Effluents from two tertiary STPs, located in the interior of BC, were evaluated for their effects on Chinook salmon (*Oncorhynchus tshawytscha*). The objectives of the study were to determine: 1) if effluents cause alterations to osmoregulatory or endocrine parameters, and 2) the amount of estrogenicity contributed by estrogen hormones. Juvenile Chinook were exposed to 0%, 1% and 5% sewage, or matching levels of constituent pure estrogens, for 7 days and then transferred to clean water for a 7-day depuration period. Tissues were collected at day 7 and day 14 (recovery), as well as after 24-hour seawater challenges that occurred at day 8 or day 15. Significant changes were found in gill ATPase activity, plasma cortisol, and estrogen-responsive gene transcript levels. One of the effluents had no detectable estrogen hormones, while the other contained 35 $ng \cdot L^{-1}$ estrone (in the 100% STP effluent). Estrogen-responsive gene transcript results were used to determine that the overall estrogenicity of the estrone-containing effluent was equivalent to that of pure estrone alone. Thus, this study demonstrates that BC STP effluents can be estrogenic, affect other physiological systems including osmoregulation, and that estrogen hormones may account for the total estrogenicity.

Evaluation of treatment efficacy for the reduction of chemicals and environmental impacts of wastewater (PL)

PILEGGI, V. [1]***, TABE, S.*** [1]***, PARROTT, J.*** [2]***, NOWIERSKI, M.*** [1]***, KLEYWEGT, S.*** [1] ***and FLETCHER, T.*** [1]

[1]*Ontario Ministry of Environment,* [2]*Environment Canada*

The Ontario Ministry of Environment partnered with the federal government, municipalities, and academia to complete a multi-faceted wastewater project. The purpose of the study was to evaluate the occurrence of various contaminants in treated sewage effluents and their reduction by eight different treatment technologies. In addition, the environmental impact of treated sewage effluent through standard acute

and chronic toxicity tests and *in-vitro* microscale tests was assessed. A baseline study was carried out in 2010 to understand the quality of effluent from two full-scale sewage treatment plants (STPs) using conventional activated sludge with nitrification (CAS-N). The study continued in 2011 (Phase 1) when different treatment technologies were evaluated in pilot plants as well as a full-scale advanced sewage treatment plant. Three separate plants comprising a total of eight different treatment technologies were evaluated. Results of the baseline study will be presented, as well as preliminary results from the Phase 1 study.

Polycyclic aromatic hydrocarbons (PAHs) in waters of the Athabasca oil-sands region: Regional distributions and correlations with landscape and other water quality variables (PL)

DAVIES, M. [1]***, JOHNSON, J.*** [1]***, WADE, K.*** [1] ***and GEE, J.*** [1]

[1]*Hatfield Consultants*

In 2011, the Regional Aquatics Monitoring Program (RAMP) assessed ambient water quality at 52 locations in streams and lakes in the lower Athabasca oil-sands region, including seasonal sampling at some locations. Samples were analyzed for over 100 water quality variables, including conventional variables, major ions, total and dissolved metals, petroleum hydrocarbons, and parent and alkylated PAHs using ultra-trace detection limits (approximately 0.1 $ng \cdot L^{-1}$). Spatial and temporal distributions in total PAH concentrations and PAH-species profiles will be discussed, as well as correlations of PAH concentrations and species-signatures with other water quality variables, hydrometric variables, and upstream landscape variables such as extent of oil-sands development and extent of surficial expression of the bitumen-containing McMurray Formation.

Toxicity of oil sands naphthenic acid extracts to early-life stages of fathead minnows (PL)

MARENTETTE, J. [1]***, FRANK, R.*** [1]***, HEWITT, M.*** [1]***, HEADLEY, J.*** [1] ***and PARROTT, J.*** [1]

[1]*Environment Canada*

Naphthenic acids (NAs) are major toxic constituents of oil sands process-affected water and thus a primary concern for the ultimate remediation of tailings ponds in Northern Alberta's oil sands regions. Fathead minnow embryo-larval survival, growth, and behaviour were studied after exposure to NA extract (NAE). NAs were extracted from fresh OSPW collected in 2009 from the Athabasca oil sands region. Fathead minnow embryos less than 24 hours old were reared in NAE ranging from 0 to 20 $mg \cdot L^{-1}$ through to 15 days post-hatch (dph), in 1 L beakers for a 21-day exposure. Larvae were assessed at 8 and 15 dph for effects on growth and behaviour (activity, rate of feeding and startle response). NAE affected larval mortality but not hatching at 20 $mg \cdot L^{-1}$ (LC_{50} = 15 $mg \cdot L^{-1}$), and few effects on larval size or behaviour were noted. To more closely assess effects of NAE on eggs, embryos < 24 hours old were exposed to NAE ranging from 0 to 80 $mg \cdot L^{-1}$ until hatch in 24-well tissue culture plates. Length at hatch, heartbeat and flexure rates at ages 1 to 3 days post-fertilization were assessed. NAE

reduced heartbeats, tended to affect flexure frequency, extended time to hatch, and ultimately decreased hatching success (LC_{50} = 14 $mg \cdot L^{-1}$), but showed no effects on hatch length, in this fish embryo toxicity test. Possible sources for the variation in effects on hatching success between the two types of early-life stage toxicity tests, as well as comparisons to commercial NA mixtures, are explored.

Larval fish toxicity of snow melt waters from oil sands areas (PL)

PARROTT, J. [1]***, NORWOOD, W.*** [1]***, GILLIS, P.*** [1]***, HEADLEY, J.*** [1]***, HEWITT, M.*** [1]***, KIRK, J.*** [1]***, FRANK, R.*** [1]***, MARENTETTE, J.*** [1]***, MCMASTER, M.*** [1]***, MUIR, D.*** [1] ***and WANG, Z.*** [1]

[1]*Environment Canada*

Embryo-larval fathead minnows were used to assess the toxicity of snow melt samples collected in the vicinity of the Canadian oil sands process facilities along the Athabasca River in the area of oil sands development and upstream. Snow samples were collected early March 2011 and 2012, and then shipped frozen back to the laboratory. Because the snow melt waters were low in essential ions, they were amended with several salts to mimic major ion levels up to those observed in the Athabasca River. Fertilized fathead minnow eggs were exposed (through hatch to 7-15 days post-hatch) to 25%, 50% and 100% amended snow melt waters. Snow samples from upstream or far downstream of the oil sands were not toxic to larval fathead minnows at 100%. Three snow samples from around the oil sands mining and refining areas were toxic to larval minnows at 25-100%. There was very little toxicity in larval mussels or *Hyalella* exposed to snow melt. Although pure melted snow caused effects in larval fish, samples of Athabasca River water collected just after snowmelt in 2010 caused no effects. Snow was analyzed for metals, naphthenic acids, and PAHs. Compared to other sites, the most toxic snow melt samples had higher concentrations of C1-C4 polycyclic aromatic hydrocarbons and metals, most likely from airborne deposition.

Population performance of white sucker *(Catostomus commersonii)* downstream of major Athabasca oil sands developments (PL)

ARENS, C. [1]***, HOGAN, N.*** [2] ***and VAN DEN HEUVEL, M.*** [1]

[1]*University of Prince Edward Island,* [2]*University of Saskatchewan*

Development within the Athabasca oil sands deposit has expanded rapidly in recent years and there are growing concerns regarding current and future impacts on fish health in the Athabasca watershed. The objective of this research was to compare the population performance of 20 male and 20 female white sucker (*Catostomus commersonii*) collected at the confluence of the Muskeg River, downstream of major oil

sands developments, with Calling Lake, a reference site far removed from the Athabasca oil sands deposit or other sources of contaminants in the watershed. Fish sampled during the May 2012 spawning migration at the Muskeg River showed evidence of exposure to organic contaminants relative to the Calling Lake site with significant EROD induction observed in both males (15-fold) and females (11-fold), as well as significantly higher bile fluorescence at the phenanthrene wavelength (5-fold). The Muskeg River fish were also significantly larger and heavier and exhibited higher condition and liver size, while reproductive endpoints, gonad size and fecundity, were significantly lower. No differences were observed in spleen size, white blood cell absolute counts or fin erosion. Based on our results fish collected downstream of development showed a response pattern consistent with exposure to hydrocarbons with potential metabolic disruption, which may result in impaired reproductive capabilities. However, due to limitations in this study design it is not possible to identify causative factors responsible for these effects, or to separate the relative contributions of oil sands development from natural erosion of oil sands material.

Terrestrial ecotoxicity of canola and tallow-biodiesel blended with ultra-low sulphur diesel (PL)

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Biodiesel is becoming an interesting alternative fuel source considering the continuously diminishing petroleum resources. Biodiesel can be produced from either plant or animal feedstocks, and its storage, transport, and use may pose potential environmental problems to soil and groundwater. Aquatic toxicities of diesel and biodiesel have been extensively studied, but little is still known about their toxicities and environmental fate in soil. The present study evaluates the potential environmental impacts of biodiesel and petroleum ultra-low-sulphur based diesel (ULSD) blended with biodiesel in soil using direct contact toxicity assays. Canola-source biodiesel (B100) and a tallow-source biodiesel (B100) alone and as 20% (volume/volume) blends with ULSD (B20) were compared to neat ULSD. Selected standard terrestrial toxicity tests included ryegrass *Lolium perenne* seedling emergence and growth, earthworm *Eisenia fetida* survival, and soil microbial dehydrogenase activity (DHA). Soil loading was quantified using extractable n-alkanes (C10 to C34 hydrocarbons). Results indicate that both B20 biodiesel blends had less deleterious effects to ryegrass growth and earthworm survival than the petroleum ULSD, whereas both B100 biodiesel samples had no deleterious effect on ryegrass growth or earthworm survival at concentrations up to 6310 and 6613 mg n-alkanes·kg $^{-1}$, respectively. Petroleum ULSD and B20 canola biodiesel inhibited the DHA microbial activity, whereas tallow biodiesel (B20 and B100) and B100 canola biodiesel stimulated the DHA activity. This study provides useful toxicological data for

the evaluation of potential adverse impact and risk assessment of biodiesel in the environment.

In vitro assessment of AhR-mediated activity and steroidogenic potential of fractions from oil sands process-affected water (PO)

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Extraction of oil from oil sands produces large volumes of process-affected water (OSPW) and there is concern surrounding the toxicity of its constituents, especially the water soluble organic fraction referred to as naphthenic acids (NA) and polycyclic aromatic hydrocarbons (PAH). However, the biological activity of these mixtures of compounds is difficult to assess. The goal of this study was to use *in vitro* assays to determine if specific fractions obtained from OSPW possess biological activity. NAs were extracted in bulk from OSPW using acid precipitation and then fractionated. Concentrations of selected chemicals were identified and quantified by use of mass spectrometry, nuclear magnetic resonance, and attenuated total reflectance infrared spectroscopy. Each fraction contained carboxylic acid groups and carbon chains typical of NAs although there were differences in average carbon number and hydrogen deficiency between the samples. The four fractions were examined for AhR-mediated activity using the H4IIE–luc bioassay and for effects on production of steroid hormones by use of the H295R steroidogenesis assay at concentrations ranging from 0.005 to 5 $mg \cdot L^{-1}$. The fraction derived from dichloromethane back extraction of base-solubilized NAs elicited AhR-mediated activity after 24 hours at 5 $mg \cdot L^{-1}$ but not after 48 or 72 hours. None of the fractions affected the synthesis of steroid hormone at the concentrations tested. Taken together, the results of the spectroscopic analysis and *in vitro* assays suggest that compounds capable of activating AhR-mediated pathways are present in OSPW, specifically in the dichloromethane fraction. The identity of the AhR-active compounds remains to be determined.

Comparing deformities in fresh and preserved cutthroat trout *(Oncorhynchus clarkii)* swim-up fry exposed to selenium (PL)

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[1]*University of Manitoba,* [2]*Stantec Consultants*

Analysis of deformities in larval fish at the swim-up stage of development, when the yolk has been almost completely assimilated and prior to exogenous feeding, is the most sensitive endpoint for evaluating the potential effects of selenium in exposed fish populations. Typically, larval fish are anaesthetized using an overdose of tricaine methanesulphonate (MS-222), preserved in buffered formalin or Davidson's solution, and subsequently analyzed for deformities in several categories, most notably spinal curvatures, craniofacial defects and edema of the yolk sac and/or pericardium. Recently, it has been suggested that at least a subset of larval fish be assessed before fixation to minimize the potential for artefacts related with the fixation process to be mischaracterized as deformities. Here we present the comparative analysis of deformities in larval cutthroat trout fry using freshly anaesthetized samples and compare the incidence of spinal, craniofacial and edema endpoints with those identified in fixed specimens. Regression analysis indicates similar results in both the proportions of identified categories of deformities and benchmark Se thresholds for those deformities.

Selenium bioaccumulation in bird eggs: Let's get cracking! (PL)

CESH, L. [1]***, DWYER, J.*** [1] ***and DEBRUYN, A.*** [1]

[1]*Golder Associates*

Coal mining has resulted in selenium releases into downstream aquatic habitats, and this represents a risk to aquatic-feeding birds that breed in these areas. Maternal transfer of dietary selenium into developing eggs can result in deformity or embryo death when egg selenium concentrations exceed effects thresholds. To support risk assessment and management of selenium, we studied the bioaccumulation of selenium in the eggs of three aquatic-feeding bird species. We collected and compiled paired measurements of water and egg selenium concentrations in American dippers (*Cinclus mexicanus*), spotted sandpipers (*Actitis macularius*), and red-winged blackbirds (*Agelaius phoeniceus*) in coal-mining regions of British Columbia and Alberta. We then used piecewise regression analysis to derive statistical bioaccumulation models. These three species exhibited distinct patterns of bioaccumulation, which may be related to differences in feeding strategies and life histories.

Exploring a mechanistic approach to understanding selenium accumulation in aquatic food webs (PL)

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Risk to aquatic organisms associated with selenium exposure has been primarily associated with dietary-based body burdens in egg-laying vertebrates. However, accumulation of inorganic dissolved selenium into the food web exhibits considerable variability among sites, which confounds the assessment of environmental risk of dissolved selenium. While much research has been conducted on the transport, transformation and effects of selenium in the environment, a broadly applicable approach that links dissolved inorganic selenium concentrations to predicted biological effects has not yet been developed. The purpose of this study was to evaluate site-specific factors that may influence the accumulation of inorganic selenium at the base of the food web. To better characterize the uptake of selenium into freshwater food webs, we explored the uptake of inorganic selenium into two species of plants (a floating aquatic macrophyte (*Lemna minor*) and a freshwater alga (*Pseudokirchneriella subcapitata*), and a planktonic crustacean (*Daphnia magna*). In one or more of these species, the effects of speciation, and interactions with sulphate, chloride, water hardness and nitrate in relation to total selenium uptake were investigated. The results of these tests provide useful input variables that might ultimately be used to develop a predictive model to estimate the uptake of inorganic selenium under differing water quality conditions.

A statistical evaluation of selenium bioaccumulation in fish (PL)

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The most reliable basis for assessing selenium risk is the use of tissue-based effects benchmarks. Tissue residue guidelines therefore have a central role in the regulation of selenium in the Canadian environment. However, selenium management is more practically based on monitoring and mitigation of aqueous selenium concentrations. Water quality guidelines therefore serve an important supporting role. The development of water quality guidelines requires an understanding of selenium bioaccumulation, to derive protective water selenium concentrations from tissue-based effects data. To support this derivation, we have undertaken a statistical analysis of selenium bioaccumulation in fish. We compiled 3,723 paired tissue and water selenium concentrations for 38 fish species from Canada and the USA. In many cases we were

also able to obtain related fish characteristics (e.g., age, size), habitat characteristics (e.g., lentic vs. lotic) and water quality characteristics (e.g., sulphate, conductivity). Our analysis provides an evaluation of the variability in selenium bioaccumulation within and among species, between habitats, and as a function of characteristics of the fish and its environment.

The effect of pH and dissolved organic carbon on the acute toxicity of cobalt to *Hyalella azteca* (PL)

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The federal Chemicals Management Plan is currently evaluating cobalt-containing substances as they have been identified as high risk to the environment and human health. In aquatic systems, cobalt (Co) speciation is impacted by measurable water quality parameters such as pH and dissolved organic carbon (DOC) concentration. Metal speciation is a major factor in metal bioavailability and toxicity to an organism.

The impact of water chemistry on the waterborne toxicity of Co to the freshwater amphipod *Hyalella azteca* was examined in 7-day acute tests with 2-9 day old animals in 400 mL synthetic soft water with mortality as the endpoint. Water chemistry parameters were adjusted to pH 6.5, 7.7, and 8.3 and nominal DOC concentrations of 2, 5, and 10 mg $C \cdot L^{-1}$. Based on measured Co water concentrations, the LC_{50} of Co to *H. azteca* decreased with increasing pH. The decreased toxicity at lower pH could be due to increased competition with H+ ions for binding sites on the biotic ligand. Higher concentrations of DOC had a protective effect and reduced the calculated LC_{50} values of cobalt. DOC can bind up free metal ions, which would reduce bioavailability. Future work includes determination of the effect of water chemistry on the chronic (4-week) bioaccumulation and toxicity of Co to *H. azteca*.

Does cadmium interact with UV radiation in altering physiological stress response, club cell investment and alarm cue production in fathead minnows *(Pimephales promelas)*? (PL)

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Recent anthropogenic activities have depleted the stratospheric ozone layer, resulting in a global increase in ultraviolet radiation (UVR). Cadmium (Cd) is an ubiquitous aquatic pollutant and is known to be toxic to aquatic organisms at extremely low concentrations. The skin of many fishes contains large epidermal club cells (ECCs)

that are known to release chemicals (alarm cues) that warn other fishes of danger. This study investigated the effects of *in vivo* UVR exposure to fathead minnows *(Pimephales promelas)*, both in the absence and presence of waterborne Cd. Specifically, we examined ECC investment, physiological stress responses and alarm cue production in fish. We found that fish exposed to UVR, either in the presence or absence of Cd, showed consistent decrease in ECC investment compared to non-exposed controls. However, the combined exposure of UVR and Cd reduced cortisol levels relative to that in UVR only exposure. Surprisingly there was no difference in the potency of the cues prepared from the skin of UVR and Cd exposed or non-exposed fish, indicating that UVR and Cd exposure combined may have little influence on chemically-mediated predator-prey interactions.

Effects of short-term and lifetime exposure to metals on the olfactory system of wild yellow perch *(Perca flavescens)*: Gene expression, neurophysiology, and behaviour (PL)

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Chemical communication mediates many essential processes in fish. Exposure of fish to low concentrations of metals has been demonstrated to impair chemical communication systems. Most studies focusing on olfactory toxicity have been performed under laboratory conditions using model species and short–term exposures to single metals. Studies using wild animals under chronic exposure conditions from metal-contaminated systems are relatively rare. To investigate the effects of both short- and long-term metal exposure, wild yellow perch from one clean and two metal-contaminated lakes were collected in and around Sudbury, Ontario. We compared the effects of copper (Cu) and nickel (Ni) exposure at three levels of biological organization. A novel yellow perch microarray was used to measure differential gene expression in olfactory tissue; electro-olfactography (EOG) to measure neurophysiological responses; and anti-predator assays to measure behavioural responses to alarm cues. Reference fish exposed to Cu or Ni for 24 hours demonstrated reduced EOG response to standard chemosensory cues relative to controls. However, differences in gene expression were detected only in the Cu-exposure; Ni-exposed fish showed no differential gene expression relative to controls. Fish from metal-contaminated lakes demonstrated lower EOG responses to standard chemosensory cues relative to those from the reference lake. However, this neurophysiological difference was not reflected in baseline gene expression. Behavioural assays corresponded with the EOG measurements; fish from metal contaminated lakes had impaired responses to alarm cues. Although impaired chemosensation was clearly detected in fish from metal-contaminated lakes using behavioural and neurophysiological assays, gene expression profiles did not reflect olfactory dysfunction.

Environmental effects monitoring: Reporting metal mines' observed effects (PL)

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[1]*Environment Canada*

Environment Canada has recently published the report "Second National Assessment of Environmental Effects Monitoring Data from Metal Mines Subjected to the *Metal Mining Effluent Regulations*", which summarizes the analyses of data from studies of fish populations, benthic invertebrate communities and sublethal toxicity test results based on information submitted to Environment Canada by regulated mines.

This presentation will focus on the summarized mine-by-mine study results. Observation of results from two consecutive studies on an individual mine basis shows that, at present, 32 mines have confirmed effects for the benthic invertebrate community, and 28 mines have confirmed effects for fish. These mines are expected to conduct investigation of cause studies. A large number of mines will continue to conduct standard monitoring.

Review of Environment Canada's "Second National Assessment of Environmental Effects Monitoring Data from Metal Mines Subjected to the *Metal Mining Effluent Regulations*" (PL)

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[1]*Stantec Consulting*

Environmental Effects Monitoring (EEM) administered under the *Metal Mining Effluent Regulations* has now been completed for two national assessment cycles of monitoring, and Environment Canada has just released its Second National Assessment of the EEM data. The assessment used the effect size statistic Hedges' d, which is based on distances between means, to summarize and interpret the EEM data for fish and benthic invertebrates. The Second National Assessment concluded that measurable environmental impacts have been caused by the mining industry and that, "*...fish collected in areas exposed to effluent were, on average, older, thinner and slower growing, with smaller livers and with more of a tendency to reduced gonad size*'", and that the benthic invertebrate community showed "*... significantly reduced taxon richness*" and "*...different groupings of benthic invertebrates in exposure areas compared to reference areas.*" The purpose of the following review was to determine if the above concluding statements were supported by the meta-data presented within the Second National Assessment report. Examination of the meta-data indicated that six of the nine endpoints showed inconsistent results between the two monitoring cycles, that many of the differences were not significant in one or both cycles, and that all but

one of the nine endpoints was consistently below biologically meaningful effects benchmarks. Review of the meta-data clearly indicated that the findings of the Second National Assessment were not supported by the data; methods prescribed by Environment Canada in its own EEM Guidance Document were not followed, statistical first principles were not considered, and development of appropriate biological benchmarks was not undertaken.

Paleoecotoxicology: Reconstructing the historical effects of contaminants and other stressors in aquatic ecosystems (PO)

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[1]*University of Saskatchewan*

For various reasons, historical (including pre-operational) environmental data are often lacking for lakes affected by industrial, municipal and agricultural activities. Without long-term data, managers are not able to show how much a system has degraded (or recovered), determine thresholds for undesirable consequences in ecosystems, determine the direction of environmental change, or set realistic mitigation goals. Aquatic sediments contain a tremendous amount of information that, once interpreted, can help fill these knowledge gaps. Stratigraphic analysis of depositional sediments is commonly used in paleobiogeography to study temporal changes in the distribution of taxa, in paleoecology to reconstruct historical ecosystems, and in paleolimnology to infer past environmental conditions in inland lakes. Depositional sediments can also be used in paleoecotoxicological investigations to reconstruct the timeline of contamination and toxicant-induced changes in aquatic ecosystems. Using Ross Lake (Manitoba, Canada) as a test case, we discuss various tools useful in reconstructing the ecological effects of almost 80 years of mining, metallurgical and municipal activities on this northern Canada lake. Physicochemical variables (e.g., trace metals, stable isotopes) and subfossil remains (diatom, chironomid, cladoceran, chaoborid) will be discussed as lines of evidence.

Assessing and managing sediment contamination in transitional waters (PL)

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Sediment contamination remains a global problem, particularly in transitional waters such as estuaries and coastal lagoons, which are the recipients of chemicals from multiple near- and far-field sources. Although transitional waters are highly productive ecosystems, approaches for assessing and managing their sediment contamination are not as well developed as in marine and fresh waters. Further, although transitional waters remain defined by their variable and unique natural water quality characteristics, particularly salinity, the biota inhabiting such ecosystems, once thought to be defined by Remane's "paradox of brackish water", are being redefined. The purpose of the present paper is to build on a similar but now dated (> 12 years old) review of methods to assess sediment contamination in estuaries, extending this to all transitional waters, including information on integrative assessments and on management decision-making. The following are discussed in addition: chemical assessments; biomarkers; bioindicators; and biological surveys. Assessment and management of sediment contamination in transitional waters needs to be focused on ecosystem services and, where appropriate and possible, needs to be proactive rather than reactive when uncertainty has been suitably reduced.

The role of sediment characteristics in the bioavailability of sediment-associated uranium to the freshwater midge, *Chironomus dilutus* (PL)

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[1]*University of Saskatchewan*

Current sediment quality guidelines (SQGs) developed for use in evaluating metal and radionuclide contamination associated with uranium (U) mining in northern Saskatchewan use total bulk concentrations of metals found in the sediment as a predictor of benthic community impairment. The number of false-positives obtained with this approach suggests that alternative approaches should be considered. Thus, the development of SQG approaches that incorporate metal bioavailability have been recommended. The dissolved phase of U is hypothesized to be the primary bioavailable U fraction to aquatic organisms, so knowledge regarding key sediment properties that influence metal distribution between the solid and aqueous phases is important for enhancing SQGs. Metals, such as U, tend to accumulate in sediments; however, there is

limited information regarding the toxicity and bioavailability of U to benthic invertebrates exposed to U contaminated sediment. Thus, this research focuses on quantifying sediment characteristics that influence bioavailability and hence toxicity of sediment-associated U to a model freshwater benthic invertebrate, *Chironomus dilutus*. A series of 10-day sediment bioaccumulation tests was employed to examine sediment characteristics (i.e., clay and organic matter content) individually, while maintaining other variables constant, to determine their influence on U bioavailability. Test endpoints and measurements included midge survival and growth, and U concentrations in organisms, bulk sediment and water (overlying and pore water). Bioaccumulation of U in *C. dilutus* larvae exposed to U-spiked sediments (50 $mg \cdot kg^{-1}$ d.w.) decreased from 1195 to 10 $mg \cdot kg^{-1}$ d.w. when clay content increased from 0 to 60%. Similar results were obtained with higher organic matter content, demonstrating that with similar total concentrations of U in sediment, the actual amount of U available for biological uptake is strongly influenced by the composition of the sediment.

A weight-of-evidence approach to investigate the fate and effect of metals in Athabasca River sediments on invertebrate and fish growth and survival (PL)

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[1]*Natural Resources Canada, Canmet MINING*

There has been much debate as to the extent of the impact on the environment from the activities conducted in the development and processing of oil sands in the Athabasca River basin. Although most research has focused on the impact of organic contaminants such as hydrocarbons, more recently published work has highlighted metals as a potential environmental concern from oil sands processing. In response to this concern, this project was conducted using a weight-of-evidence approach to gain a better understanding of metal fate and effects within the Athabasca River region. This presentation outlines the assessment on sediments collected at various points along the river to assess both fate and effect of metals. The use of mini-peepers and diffusive gradients in thin films (DGTs) was employed alongside toxicity tests using the benthic invertebrate *Chironomus dilutus* and the freshwater fish *Pimephales promelas*. This allowed the comparison of differing techniques to assess both pore-water and overlying water metal concentrations and compare with effect data and tissue concentrations in the organisms tested. Comparison with DGT and peeper data show that concentrations of metals in pore waters were generally higher than in surface water and in sites adjacent to oil sands activity. However, no effects on survival or growth were observed in either the invertebrate or fish species tested. Metal concentrations were elevated in the benthic invertebrate but not in the larval fish data, indicating that metal releases from sediments into the overlying water were minimal. Of the two techniques used to

assess metal concentrations, DGTs showed the strongest correlation with tissue concentrations compared to peepers. The use of multiple techniques allowed a better understanding of metal fate within these sediments.

What's that smell? A sediment porewater TIE implicates sulphide (PL)

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In a recent investigation of sediment quality for a harbour planned for re-development, a toxicity identification evaluation (TIE) of the cause of porewater toxicity to the echinoderm, *Strongylocentrotus purpuratus*, was an important consideration in development of a preliminary sediment management strategy. Metals and PAHs concentrations in sediments exceeded federal and provincial benchmarks, but at many stations metals and PAH bioavailability appeared to be limited. Samples were not toxic to photoluminescent bacteria but a small number of samples elicited a 20-30% reduction in amphipod survival and polychaete growth, possibly due to bioavailable metals or PAHs. In contrast, toxicity to echinoderm fertilization success was variable and did not correlate to sediment contaminant concentrations. A porewater TIE involving sulphide purging and metals chelation suggested that the toxicity was not due to contaminants identified in Harbour sediments. Rather, potential aluminum and/or sulphide toxicity was implicated, with sulphide being the more likely toxicant based on correspondence between observed sulphide concentrations, echinoderm toxicity, and known toxicity thresholds. The *Disposal at Sea Regulations* of the *Canadian Environmental Protection Act, 1999* prescribes consideration of toxicity test results for amphipod, photoluminescent bacteria and echinoderm for decision-making with respect to Disposal at Sea permitting. Given that elevated sulphides in sediment porewater were the result of natural decay and redox conditions in the sediments rather than sulphur contamination, it is proposed that the finding of echinoderm toxicity should not preclude Disposal at Sea for sediments that were not toxic to the other test species.

The reference envelope: Technical basis for assessing toxicity in contaminated sediments (PL)

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The reference envelope (RE) is an alternative approach to assessing sediment toxicity intended to overcome limitations imposed by the use of control sediments whose non-contaminant characteristics may influence toxicity and contribute to low

statistical power when test sediments are compared to a single control. In the RE approach, test sites are classified as toxic if they fall below the lower 5th centile of control-adjusted toxicity endpoints of the reference sites (RS), selected to have low concentrations of chemicals (*Hyalella azteca*) and 70% (*Chironomus dilutus*); 2) PECQ *H. azteca*) and 0.60 (*C. dilutus*) mg/individual. The number of sites classified as reference declined by ~20% when the PECQ criterion was reduced from 0.2 to 0.1; increasing the survival criterion or adding a weight criterion had little influence on the number of sites classified as reference and therefore on the RE value. Using median-adjusted versus control-adjusted data did not influence the number of sites classified as toxic. These data indicate that incorporating stricter criteria for RS selection has little influence on test site classification.

Toxicity of the sea lice pesticide deltamethrin to the polychaete worm *Nereis virens* (PL)

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Integrated Multi-Trophic Aquaculture (IMTA) incorporates species from different trophic levels to extract organic waste from aquaculture. Polychaete worms are efficient detritivores with high commercial value and methods for including worms under aquaculture cages have been studied on the east coast of Canada. Sea-lice infestations in salmon aquaculture necessitate treatment of fish. Fish are exposed via bath treatments or well boats to anti-louse therapeutants, which are subsequently released into the environment. As a result, other organisms may potentially be exposed to therapeutants, including IMTA extractive species. The anti-louse therapeutant Alphamax®, containing the pyrethroid deltamethrin, has been used in aquaculture under emergency approval in eastern Canada. Deltamethrin has been shown to be highly toxic to crustaceans; however, there is no toxicity data for polychaetes. The goal of this project is to determine the potential toxicity of deltamethrin to the marine polychaete *Nereis virens* in laboratory studies. Mortality was not observed in worms exposed for 24 hours to deltamethrin in sea water at concentrations up to 2 $\mu g \cdot L^{-1}$ (the aquaculture treatment concentration). Based on its chemical properties deltamethrin will likely adsorb to organic particles and thus may accumulate and persist in sediment, representing another potential route of exposure for worms. Acute and chronic studies are ongoing to determine the effects of deltamethrin spiked into sediment on worms, evaluating endpoints including survival, growth, and behaviour (e.g., lack of burrowing/sediment avoidance). These toxicity data can be used to evaluate the potential risks of deltamethrin to worms if incorporated as another extractive species at IMTA sites.

The phantom midge *(Chaoborus)* and the mystery of the disappearing fishes (PL)

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Ross Lake is a small, shallow lake located within the municipality of Flin Flon, Manitoba, Canada. The surficial sediments of Ross Lake are highly contaminated with various trace metals, the result of long-term mining and metallurgical activities. Also, the early decades of Flin Flon saw an unknown volume of raw sewage discharged into the northern basin of Ross Lake. Anecdotal evidence suggests that Ross Lake does not currently have a resident fish community. Because biophysical data were not collected from Ross Lake until well after operations began, it is unknown when fish were extirpated. Subfossil remains (mandibles) of the phantom midge, *Chaoborus*, have been used by other investigators to infer the presence or absence of fish in inland waters. Some species of *Chaoborus* can coexist with fish, while others cannot (e.g., *Chaoborus americanus*). The presence in the sediment profile of mandibles of *C. americanus* larvae can be used to date the absence of fish in Ross Lake. Sediment cores were collected from the southern basin of Ross Lake and analyzed for various physicochemical variables (e.g., trace metals, stable isotopes) and subfossil remains (diatoms, chironomids, cladocerans, chaoborids). *Chaoborus* remains were found in pre-industrial sediments, but were largely absent from post-industrial sediments. No *C. americanus* mandibles were found in any portion of the sediment profile. This suggests that Ross Lake was either directly toxic to *Chaoborus* larvae, or that *Chaoborus* larvae were indirectly impacted by food web alterations. The usefulness of *Chaoborus* remains in paleoecotoxicological investigations will be discussed.

Integration of higher level and molecular endpoints for an early life stage salmonid bioassay for monitoring polluted environments (PO)

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We examined the utility of adopting molecular endpoints for a widely used *in situ* early life stage (embryos to swim-up fry) cutthroat and rainbow trout bioassay to increase sensitivity of a diagnostic assessment of chemical stressors in the aquatic environment. In conjunction with field studies in reference and polluted urban streams in British Columbia, parallel single concentration laboratory exposure experiments were conducted with model compounds for metal (zinc), polycyclic aromatic hydrocarbons (pyrene), and endocrine disruption (estrogen). Endpoints measured in embryos included survival, deformities, size, vitellogenin (Vtg), metallothionein (Mt) protein, and gene expression assays for transcripts that are responsive to metals (Mt A/B), endocrine disruptors (vtg, estrogen receptors) and stress (cyp1a3, glutathione transferases (gst)). In the laboratory, protein Vtg was inducible with 1 $\mu g \cdot L^{-1}$ 17β-estradiol in rainbow trout fry. However there was no response in gill metallothionein protein levels after 40 $\mu g \cdot L^{-1}$ zinc sulphate exposures and few changes in gene expression after exposure to model compounds. In the field, there were no changes in protein or mRNA levels of vtg or mt; however, cyp1a3 and gstk mRNA significantly increased in fry from the polluted sites, suggesting a generalized stress response. Gene patterns in polluted streams were more similar to each other than to the reference site, demonstrating that expression profiles can distinguish between reference and polluted field sites. Although future dose-response studies are needed to assess the sensitivity of the molecular endpoints examined in the *in situ* early life stage salmonid bioassay, combining molecular and morphometric measures for use in the assessment of chemical contamination in the aquatic environment holds considerable promise for assessing impacts of environmental pollutants.

Silurana tropicalis larvae exposed to the industrial dye Disperse Yellow 7 (PO)

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Dyes used in fabric and leather industry are being released into and accumulating in Canadian ecosystems. Recent studies have demonstrated that dyes made of azo compounds significantly increased toxicity in biota, which is explained by their toxic metabolites (e.g., aromatic amines) produced. The metabolites of azo compounds interact with hydrophobic surfaces of cell membranes and expand them, which impedes normal cellular functions. The aim of this study is to evaluate the toxicity and sub-lethal effects of the azo dye Disperse Yellow 7 (DY7) in amphibians using ecotoxicogenomic approaches. Larvae of the frog *Silurana tropicalis* (Western clawed frog) were exposed to DY7 contaminated water (nominal concentrations ranging 0 – 0.8 $\mu g \cdot g^{-1}$) and contaminated sediment (0-887 $\mu g \cdot g^{-1}$). Short-term exposures were performed during early frog development from Nieuwkoop-Faber developmental stage 12 to 46 and lasted 4 days. At exposure completion, survivorship was assessed and whole larvae were sampled for further analysis. Blind analysis of malformations was performed on a subset of the samples that were preserved in 3% formalin. RNA was isolated from the second subset of samples using commercially available kits. Using real-time RT-PCR, gene expression was analyzed for the following genes: heat shock proteins 30, 70 and 90 (stress axis); thyroid hormone receptor β and deiodinase type 2 (thyroid hormone); estrogen receptor α and aromatase (female reproductive system); and androgen receptor and steroid 5 α-reductase type 2 (male reproductive system). Survivorship, malformation and biomarkers of the stress axis, of thyroid hormones, and of both the male and female reproductive systems will be presented.

Testing for DNA ploidy changes associated with cancer development in mussels exposed to municipal wastewater effluent (PO)

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The mussel *Mytilus trossulus* is a key species in marine intertidal communities on the Canadian west coast and has been used as an ecosystem health indicator species for several decades. Bioaccumulation and toxicity markers are common measurements being done to monitor stress, but they are rarely linked to health outcomes. *M. trossulus* is prone to a blood cell disorder called haemic neoplasia, which is at least in part associated with environmental conditions and which develops much faster than human cancers. Including cancer-related biomarkers into environmental monitoring programs is important to provide early warning of potential population-level changes.

We developed a new biomarker for accurate detection of the disease based on flow cytometry analysis of hemocyte ploidy. The marker is being tested on mussels exposed in laboratory tanks to disinfected effluent from a primary wastewater treatment facility. The DNA ploidy marker is being compared to genotoxicity endpoints (DNA damage and micronuclei) and haemocyte morphology alterations.

Molecular Toxicity Identification Evaluation (PL)

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Daphnia magna is a bio-indicator organism accepted by several international water quality regulatory agencies. Current approaches for assessment of water quality rely on acute and chronic toxicity that provide no insight into the cause of toxicity. Recently, molecular approaches, such as genome wide gene expression responses, are enabling an alternative mechanism-based approach to toxicity assessment. While these genomic methods are providing important mechanistic insight into toxicity, statistically robust prediction systems that allow the identification of chemical contaminants from the molecular response to exposure are needed. Here we apply advanced machine learning approaches to develop predictive models of contaminant exposure using a *D. magna* gene expression dataset for 36 chemical exposures. We demonstrate that we can discriminate between chemicals belonging to different chemical classes including endocrine disruptors, metals and industrial chemicals based on gene expression. We also show that predictive models based on indices of whole pathway transcriptional activity can achieve comparable results while facilitating biological interpretability.

Application of a gene expression approach for sediment Toxicity Identification Evaluation (PL)

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Identification of the cause of sediment toxicity is needed in order to design effective management actions to improve sediment quality. Sediment Toxicity Identification Evaluations (TIEs) based on traditional methods have limited usefulness when multiple potential stressors are present or when the magnitude of toxicity is relatively low. A potential new method for stressor identification in sediment toxicity tests has been developed that is based on measuring changes in gene expression. A custom microarray containing approximately 8,600 probes for RNA transcripts was developed from the expressed genome in the marine amphipod *Eohaustarius estuarius*, a species widely used for sediment toxicity assessment. Differential gene expression in *E. estuarius* was measured following exposure to a variety of toxicants, including chlorinated pesticides, metals, current use pesticides, and PAHs. Distinctive patterns of gene expression were identified for most contaminants, suggesting that development of

a diagnostic tool for stressor identification is feasible. A set of diagnostic genes and a classification model focused on pyrethroid pesticides was developed using multivariate statistical methods. Initial application of the approach as a TIE tool using independent validation samples from lab and field studies was successful, with the model correctly identifying the primary cause of toxicity to *E. estuarius*. Use of gene expression is a promising tool for sediment TIE.

Nanosilver effects on *Xenopus laevis* tadpole metamorphosis at environmentally-relevant concentrations (PL)

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Nanosilver (nAg) is one of the most prevalent nanoparticles in consumer and industrial use today, and therefore has many routes for environmental contamination. It is predicted that low concentrations will be found in bodies of water that sustain aquatic species, including amphibians during sensitive larval stages and throughout metamorphosis into adults. Metamorphosis is a complex developmental process driven by thyroid hormone (TH) where tadpoles are sensitive to stimuli such as pollutants or endocrine disruptors. In this study, the effects of low, environmentally relevant nAg levels on *Xenopus laevis* tadpoles during metamorphosis are examined with a 28-day chronic static-renewal exposure. Deviations in developmental progression throughout the exposure were monitored and nAg body burden was determined at the conclusion of the study. Furthermore, gene expression changes after 2 days were examined with microarray and QPCR technology. The morphometrics were not significantly altered due to nAg exposure at any concentration, in contrast to earlier studies with the native bullfrog species. However, several genes responded strongly and could be considered for use as biomarkers of low-level nAg exposure. These results show differential species sensitivities, and emphasize the importance of a comprehensive evaluation of the sublethal effects on various aquatic species.

Novel bioinformatics methods to exploit omics data in fish (PL)

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Omics technologies (transcriptomics, proteomics, metabolomics) are being used as high-throughput screening tools for toxicity testing. There is evidence that molecular responses can be used to (1) classify aquatic pollutants (i.e., molecular fingerprinting), (2) provide insight into chemical mode of action (MOA) and (3) may become predictive for adverse effects (adverse outcome pathways). Many different algorithms are

regularly used to extract valuable biological information from omics data. However, many algorithms used by researchers are not centralized and cannot provide further information beyond their initial application. Using examples from fish, we present emerging bioinformatics approaches to analyze omics data. We provide proof of concept that algorithms such as Support Vector Machine learning are able to classify herbicides into androgenic or anti-androgenic MOA based on transcriptomics data from multiple fish species. In addition, we demonstrate that sub-network enrichment analysis (SNEA) offers a powerful approach for mapping regulatory networks of genes and proteins after exposures to chemicals that affect androgen receptor signaling. Connectivity between molecules is generated from relationships (i.e., expression and regulation), providing novel insight into the cell pathways affected by pollutants. Lastly, we leverage Semantic Automated Discovery and Integration (SADI) to exploit fish ecotoxicogenomics data. We have developed >40 SADI services that can be combined in multiple ways to answer queries about chemicals and molecular responses. We provide a case study with dieldrin, an organochlorine pesticide that is associated with neurodegeneration. As omics applications in ecotoxicology increase, bioinformatics support will play an increasingly significant role in data management and analysis.

Gene expression analysis of rainbow trout exposed to ibuprofen: Highlighting the benefits and challenges of using RNA-Seq on non-model organisms (PL)

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Recent advancements in next-generation sequencing have dramatically revolutionized "-omics" studies. RNA-Seq has since emerged as an important and versatile tool for characterizing transcriptomes. However, due to challenges in costs and bioinformatics requirements, the application of RNA-Seq in the field of ecotoxicogenomics is still relatively novel. In this proof-of-concept study, we evaluate the feasibility of using RNA-Seq and pathway-based analysis to examine the transcriptomic response of aquatic organisms exposed to environmental contaminants. Because RNA-Seq is not limited by the availability of genomic sequence information, it is advantageous for non-model species such as rainbow trout (*Oncorhynchus mykiss*), which are used routinely in ecotoxicology studies but currently lack a reference genome sequence. Ibuprofen is one of the most abundant pharmaceuticals found in sewage and surface water; it is known to affect prostaglandin synthesis in humans but less is known about its effects on fish. Underyearling rainbow trout were exposed to environmentally relevant, and higher, concentrations of ibuprofen for 96 hours and paired-end Illumina short read sequencing was conducted on fish liver mRNA. Differential gene expression analysis was examined at the gene and pathway level. Both expected and novel gene expression changes were observed in rainbow trout in response to ibuprofen, findings

subsequently validated by qPCR. As expected, there were statistically significant changes in prostaglandin pathway-associated genes. More surprisingly, alterations in oogenesis pathway-associated genes were also observed. As will be discussed, this analysis of rainbow trout RNA-Seq data highlights the benefits and challenges of using RNA-Seq on non-model organisms in the context of ecotoxicogenomics.

A blood test for frogs? Metabolomics in action to define contaminant effects (PL)

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Amphibia are regarded as sentinel species for a variety of habitats. They have been used for standard toxicity assays and are increasingly important in the assessment of sublethal effects that can occur, for example, as a consequence of endocrine disruption. Frogs are sensitive to a variety of endocrine disrupting chemicals (EDCs) and their tadpoles represent the most sensitive indicators of disruption of thyroid hormone action in vertebrates. Metamorphosis of a tadpole into a froglet requires thyroid hormone. Exogenous thyroid hormones, and mimics or inhibitors thereof, disrupt this critical period of postembryonic development that requires exquisite timing and coordination of diverse cellular, molecular, and physiological functions. To date, it has been difficult to obtain a more global overview of changes that occur in the frog tadpole that could have utility in identifying biological systems that are particularly sensitive to perturbation by environmental contaminants. We have taken a metabolomics approach to identify metabolites that are strongly associated with exposure to substances demonstrated to affect postembryonic tadpole development such as 3,3',5-triiodothyronine, nanosilver, and ionic silver. North American bullfrog blood serum metabolites were liquid/liquid extracted and analysed using direct infusion-Fourier transform ion cyclotron resonance mass spectrometry (DI-FTICR MS) and ultra performance liquid chromatography (UPLC) –MS using BEH C-8 and HSS-T3 columns. Metabolites were ionized by electrospray and detected in both positive-ion and negative-ion modes. Of the thousands of metabolite features obtained, specific subsets of metabolites were significantly altered upon chemical exposure including those in lipid, bile, and nucleotide synthesis pathways.

Molecular and morphological alterations of postembryonic development of Pacific tree frog *(Pseudacris regilla)* tadpoles by triclosan (PL)

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The Amphibian Metamorphosis Assay (AMA), developed for *Xenopus laevis*, is designed to identify chemicals that disrupt thyroid hormone (TH)-mediated biological processes. We adapted the AMA for use on an ecologically-relevant North American species, the Pacific tree frog (*Pseudacris regilla*), and applied molecular endpoints to evaluate the effects of the antibacterial agent triclosan (TCS). Premetamorphic (Gosner stage 26-28) tadpoles were immersed for 21 days in solvent control, 1.5 $\mu g \cdot L^{-1}$ thyroxine (T4), 0.3, 3 and 30 $\mu g \cdot L^{-1}$ TCS, or combined T4/TCS treatments. Exposure effects were scored by morphometric (developmental stage, wet weight, and body, snout-vent and hindlimb lengths) and molecular (mRNA abundance using quantitative real time polymerase chain reaction) criteria. T4 treatment alone accelerated development concomitant with altered levels of TH receptors α and β, proliferating cell nuclear antigen, and gelatinase B mRNAs in the brain and tail. We observed TCS-induced perturbations in all of the molecular and morphological endpoints, indicating that TCS exposure disrupts coordination of postembryonic tadpole development. Clear alterations in molecular endpoints were evident at day 2 whereas the earliest morphological effects appeared at day 4 and were most evident at day 21. The nature of the alterations suggests perturbation of TH signaling pathways. The Pacific tree frog is the most sensitive species examined to date displaying disruption of TH-mediated development by a common antimicrobial agent.

Transcriptomic effects of exposure to 17 alpha-ethynylestradiol during sexual differentiation on genetic male *Xenopus laevis* (PL)

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Genetic male African clawed frogs (*Xenopus laevis*) display feminized/demasculinized phenotypes after exposure to potent estrogens during the period of sexual determination and differentiation, but little is known about the molecular changes that drive the development of these altered phenotypes. Thus, the transcriptome-level effects of exposure to 17α-ethynylestradiol during this sensitive period were evaluated in *X. laevis* by use of Illumina sequencing coupled with RNA-Seq

expression analysis. The abundances of a subset of transcripts that were determined to be regulated by RNA-Seq were also measured by use of quantitative polymerase chain reaction (qPCR) to validate the quantitative capacity of RNA-Seq. There was a significant correlation (R^2=0.78) between the fold-change values calculated for RNA-Seq and qPCR. Overall, a number of pathways and processes were impacted by exposure to ethynylestradiol, including steroid and xenobiotic signaling and metabolism, steroid biosynthesis, thyroid hormone signaling and metabolism, and testicular development and spermatogenesis. Some of the altered pathways, such as thyroid hormone signaling and metabolism and testicular development, could be linked with observed biological effects on gonadal phenotypes and metamorphosis that were observed in a group of frogs that was exposed to ethynylestradiol throughout larval development.

Investigating the impacts of phenanthrene in female fathead minnow *(Pimephales promelas)* ovary and liver using gene expression profiling and higher level biological endpoints (PL)

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Phenanthrene (PHEN), a poly-aromatic hydrocarbon, is present in the aquatic environment due to natural and anthropogenic sources (e.g., oil deposits and urban runoff). PHEN has been shown to induce stress in teleosts, and recent studies suggest that PHEN may be an endocrine-disrupting substance. To better characterize the effects of PHEN on fish health, fathead minnows (FHM; *Pimephales promelas*) were exposed to measured waterborne concentrations (average over 24 hours) of 0, 29.75 (Low), 389.4 (Medium), and 942.5 (High) ng·mL^{-1}. FHM were sampled at 24, 48 and 72 hours. Ovarian and hepatic tissues were excised for gene expression analysis (48 hours) and higher level endpoints assessed. Microarray analysis revealed expression patterns for high PHEN were unique from control and low PHEN in liver. Several gene subsets also showed dose-responses to PHEN (e.g., c-myc and fibrillarin). Common cell processes impacted by PHEN across doses included sterol biosynthesis, cholesterol metabolism and those involved in immune function. Ovarian impacts were less pronounced. Genes involved in sex steroid receptor signaling, steroidogenesis, and the aryl hydrocarbon pathways, along with 17β-estradiol (E2) production, were not affected. In a second experiment, FHM ovary explants were exposed to 0.017, 1.78, and 178 ng·mL^{-1} PHEN *in vitro* for 12 hours. Transcripts from those processes above also showed no changes with PHEN exposure, and E2 production was not affected *in vitro*. These results suggest that PHEN exposure may not directly disrupt reproductive endpoints. Based upon these and other studies, we suggest that exposure to PHEN may initially cause a stress response in fish that indirectly leads to reproductive impairment over time.

Salmonid safety and West Nile virus mosquito control: Can they coincide? (PL)

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We investigated the potential for direct effects on juvenile Coho salmon (*Oncorhynchus kisutch*), of four common mosquito larvicides (Mosquito Dunks® and Bits® (Active Ingredient [AI] = *Bacillus thuringiensis* var. *israelensis*), VectoLex® WSP [AI = *Bacillus sphaericus*], VectoLex® CG [AI = *B. sphaericus*], and Altosid® Briquets [AI = s-methoprene]). Two separate tests were conducted with the larvicides according to USEPA guidelines for static toxicity tests. The tests determined the effect of the larvicide used 1) singly and 2) in combination. In concurrent research, the efficacy and persistence of larvicides in catch basins and outflow pipes was determined (summers, 2006-2008). All treatments resulted in a rapid and dramatic reduction (≤ 7 days) in either the number of mosquito pupae present (*B. sphaericus* and *Bacillus thuringiensis* var. *israelensis* [Bti]) or in mosquito emergence success (s-methoprene). *B. spharicus* and Bti were detected in three urban creeks connected to treated catch basins. In all cases, water samples collected from outflows and analyzed for *B. sphaericus* and Bti were orders of magnitude less than those collected from the test tanks during the 96-hour toxicity tests. At the height of concentration (ca. 24 hours into exposure), none of the fish exhibited any overt effects. These laboratory toxicity test results suggest that concentrations of *B. sphaericus* and Bti detected in each of the watersheds pose no direct hazard to juvenile Coho salmon. Laboratory toxicity tests also suggest that the maximum recommended dose of s-methoprene used in mosquito control poses no overt threat to juvenile Coho salmon.

Use of fluorescein dye to predict dispersion and potential effects of anti-louse pesticides in southwest New Brunswick (PL)

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Studies conducted at the St. Andrews Biological Station since 1993 have identified potential hazards associated with the use of anti-parasitic drugs and pesticides in the finfish aquaculture industry. From 2009 through 2011, severe infestations of sea-lice in southwest New Brunswick led to the emergency registration (ER) of several pesticide formulations: Salmosan®, a.i. azamethiphos (ER from 2009-2011), AlphaMax®, a.i. deltamethrin (ER in 2009-2010) and Paramove®, a.i. hydrogen

peroxide (ER from 2009-2011). Lethal thresholds have been determined for these pesticides over increasingly shorter time frames in hopes of simulating environmentally-relevant exposure scenarios. AlphaMax® is the most toxic of the formulations with 1-h, LC_{50}s in the $ng \cdot L^{-1}$ range, Salmosan® is lethal to lobsters in the $\mu g \cdot L^{-1}$ and Paramove® is not lethal to lobsters at environmentally relevant concentrations ($g \cdot L^{-1}$). Beginning in 2009, operational anti-louse treatments were conducted incorporating fluorescein dye to track the effluent plume. Dispersion models were developed using data collected during these treatments, and these models have enabled us to predict potential zones of impact for the three pesticides. We will report lethal thresholds and predicted areas of impact for the three pesticide formulations under two treatment scenarios. AlphaMax® may affect sensitive non-target species over an area of several hectares and at distances > 5 km from the point of release.

Examination of the fate and effects of the glyphosate adjuvant, POEA, using mesocosms in a small pre-Cambrian shield lake (PL)

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Glyphosate-based herbicides (e.g., Roundup) are used extensively worldwide. Traditional formulations contain the active ingredient glyphosate paired with the non-ionic surfactant polyoxy-ethyleneamine (POEA) to enhance foliar uptake. While few environmental effects have been attributed to glyphosate, the impacts of POEA in aquatic environments are uncertain. In this study, the fate and effects of environmentally relevant concentrations of POEA are being examined in nine shallow (1m depth) mesocosms in a small pre-Cambrian shield lake at the Experimental Lakes Area in northwestern Ontario. Three different treatments were assigned to the mesocosms. Low exposure mesocosms were treated nominally with a one-time addition of POEA at 10 $\mu g \cdot L^{-1}$, high exposures received 100 $\mu g \cdot L^{-1}$ and reference mesocosms received no POEA. Each treatment was performed in duplicate in six enclosures that were open to the sediments. Because POEA is known to have high affinity for particulates and sediments, three of the mesocosm curtains were constructed with impermeable bottoms to prevent interaction with the underlying lake sediments. Each of these was assigned to one of the treatments. POEA concentrations in abiotic and biotic compartments are being monitored in order to determine fate of the compound, and the chronic toxicity of POEA to fathead minnows, macrophytes, zooplankton and emergent invertebrates is being assessed for a period of 77 days after the additions. Preliminary results are expected to reveal a reduced half life of POEA in the water column than documented in previous studies due to the high content of total organic carbon present in one mesocosm treatment.

Common agents identified in toxicity evaluations of industrial effluents (PL)

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Investigations are commonly conducted on a variety of industrial effluents to identify toxic agents that have caused failures in acute rainbow trout and *Daphnia magna* bioassays used to evaluate permit compliance. These investigations often indicate toxicity caused by agents or compounds common across different industries such as mining, pulp and paper, wastewater treatment, and others. In our experience, the identification of these agents may be straightforward or challenging depending on the nature of the toxic agent(s). Some commonly implicated causes of toxicity include ammonia, metals, polymers, chlorine and ion imbalance. Fish and *Daphnia* often have differing sensitivities to these toxic agents. This presentation will draw upon case studies to discuss the nature and effects of these toxic agents in industrial effluents and some of the challenges involved with toxicity identification evaluations. This information may be helpful in assisting managers' understanding of toxicity issues in permitted effluents across different industries.

Hunting for mutants: DNA mutations in wild cormorants from Hamilton Harbour associated with exposure to PAH pollution (PL)

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Hamilton Harbour, Ontario, is one of the most polluted sites on the Great Lakes and is affected by airborne and sedimentary contamination as a result of both heavy vehicle traffic and emissions from steel production. Polycyclic aromatic hydrocarbons (PAHs) are chemical by-products of incomplete organic combustion; they are present at very high concentrations in both the air and sediment of Hamilton Harbour. Exposure to PAHs can cause a variety of health effects in both humans and wildlife, including reproductive toxicity, asthma, and mutations. We sampled large waterbirds, Double-crested Cormorants (*Phalacrocorax auritus*), in two colonies from Hamilton Harbour which were located at different distances from sources of PAHs and other industrial pollution. We also sampled cormorants at an island in northeastern Lake Erie, which served as a cleaner reference site for comparison. We looked for genetic effects and signatures of PAH exposure in cormorants from these three sites. Using five highly variable microsatellite regions (stretches of repeated DNA), we found DNA mutations in cormorant families from all three sites, but the majority of mutations were identified from the site closest to industrial sources of PAH pollution. We identified metabolites of the PAH benzo[a]pyrene in bile and liver samples of cormorant chicks using the

analytical chemistry technique LC-MS/MS (liquid chromatography-tandem mass spectrometry). The presence of this chemical in their internal body tissues suggests that cormorants in Hamilton Harbour are exposed to and metabolizing PAHs, highlighting their potential to have caused these mutations we observed.

Contamination of perfluorooctane sulphonate and other perfluorinated compounds downstream of an international airport, Hamilton, Ontario (PL)

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Per- and poly-fluorinated compounds (PFCs) are used in a wide variety of industrial, commercial and domestic products. This includes aqueous film forming foam (AFFF), which is used by military and commercial airports as fire suppressants. A preliminary assessment prior to this study revealed very high concentrations (> 1 $\mu g \cdot g^{-1}$ wet weight) of perfluorooctane sulphonate (PFOS) in snapping turtle (*Chelydra serpentina*) plasma collected in 2008 from Lake Niapenco in southern Ontario. In order to ascertain the source of these high PFOS concentrations, we conducted a spatial survey of water, amphipods, and shrimp in Lake Niapenco and the Welland River, downstream of the John C. Munro International Airport, Hamilton, Ontario. PFOS dominated the sum PFCs in all substrates (e.g., > 99% in plasma of turtles downstream the airport, and 72% to 94% at all other sites). PFOS averaged 2223±247 (SE) $ng \cdot g^{-1}$ in turtle plasma from Lake Niapenco, and ranged from 9.0 to 171 elsewhere. Mean PFOS in amphipods and in water were 518±84 $ng \cdot g^{-1}$ and 130±44 $ng \cdot L^{-1}$ downstream of the airport, and 19±2.7 $ng \cdot g^{-1}$ and 6.8±0.5 $ng \cdot L^{-1}$ at reference sites, respectively. Concentrations of selected PFCs declined with distance downstream from the airport. Perfluoroethylcyclohexane sulphonate (PFECHS), a cyclic perfluorinated acid used in aircraft hydraulic fluid, was also discovered in biota and water downstream the airport. Recent sampling of fish downstream the airport by the Ontario Ministry of Environment resulted in consumption restrictions due to PFOS. Although there was no documented spill event or publicly reported use of AFFF associated with a fire event at the Hamilton airport, the airport is likely a major source of PFC contamination in the Welland River.

The aryl hydrocarbon receptor signaling pathway of fishes: Implications for sturgeon sensitivity to dioxin-like compounds (PL)

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Activation of the aryl hydrocarbon receptor (AhR) regulates all effects of exposure to dioxin-like compounds (DLCs). Exposure to DLCs causes a range of adverse effects in fishes, including teratogenicity, hepatotoxicity, and reproductive impairment. Sturgeons are ancient species of fish, and due to their endangered status they are of interest for ecological risk assessment. However, little is known about the AhR signaling pathway of these or other ancient fishes. To begin characterizing the sensitivity of sturgeons to DLCs, we investigated the molecular responses of white sturgeon (WS; *Acipenser transmontanus*) to a model AhR agonist, β-naphthoflavone (βNF). WS were found to be among the more responsive of fishes with regard to inducibility of CYP1A. Nucleotide sequences from three distinct AhRs (AhR1, AhR2, AhR3) were identified for the first time in WS using Illumina® sequencing. All three identified AhRs had greatest expression in liver, gill, and heart of WS. Following exposure to βNF, AhR2 and AhR3 expression was up-regulated in liver, gill, and intestine; however, AhR1 was only up-regulated in gill. The functional significance of sturgeons expressing three AhR genes and implications for sturgeon sensitivity to DLCs are currently unclear. However, the great sensitivity of salmonids to DLCs is hypothesized to be partially driven by these species expressing multiple, functional AhR genes. Further research is necessary to investigate the toxicological significance of the unique AhR signaling pathway in WS and determine how this knowledge could be used to predict the sensitivity of other species of endangered sturgeons.

Hardness effects on the acute toxicity of sulphate, nitrate, selenium and cadmium as a mixture to *Daphnia magna* and *Hyalella azteca* (PL)

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Water hardness is thought to protect freshwater invertebrates against the toxicity of some chemical substances, such as metals. Water quality guidelines (WQG) are often based on controlled laboratory studies examining single contaminants under relatively simple exposure scenarios that may oversimplify the complexities of natural systems. British Columbia uses WQG to regulate the release of selenium (Se), cadmium (Cd), nitrates and sulphates to freshwater systems; however, it is uncertain if the proposed WQG for each individual contaminant remains protective when all four contaminants occur together under short exposure. Moreover, the effect of water

hardness on the four-way mixture toxicity is unknown. In this study, *Hyalella azteca* and *Daphnia magna* were exposed for 48 hours to a mixture of selenium, cadmium, nitrate, and sulphate or each contaminant individually at concentrations that corresponded to the proposed short term BC and Canadian WQG for each individual contaminant in soft (50 $mg \cdot L^{-1}$ as $CaCO_3$) and hard (300 $mg \cdot L^{-1}$ as $CaCO_3$) water. During single-contaminant exposures at proposed WQG concentrations, Cd showed significant toxicity to *D. magna* in hard water. Although Cd showed elevated toxicity to *D. magna* in soft water, the difference from the control was not statistically significant. *H. azteca* showed no significant toxicity to any of the four individual contaminants at proposed WQG concentrations in both hard and soft water. The four-way mixture showed no significant toxicity to either *D. magna* or *H. azteca* in both hard and soft water. For *D. magna* in hard water, Cd toxicity was mitigated in the presence of elevated Se.

Canadian Water Quality Guidelines for uranium should consider modifying factors (PL)

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The 2011 Canadian water quality guidelines (CWQGs) for uranium do not consider toxicity modifying factors. However, more recent data than considered in those CWQGs provide further evidence that dissolved organic carbon (DOC) reduces uranium bioavailability and toxicity. Determinations of site-specific uranium water quality benchmarks must consider the modifying effects of DOC as well as other factors including hardness and pH; when predictions of uranium toxicity based on water quality parameters are possible, the uranium CWQGs will need to be revised. The modifying effects of DOC (and other key physico-chemical variables) have implications for regulatory permitting, effluent management or treatment, and decommissioning.

Development of a site-specific water quality objective for nitrate for the EKATI diamond mine (PL)

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A site specific water quality objective (SSWQO) was developed for nitrate for BHP Billiton's EKATI diamond mine, based on chronic toxicity test data. Acceptable toxicity data were available for one alga, four invertebrates and four fish, meeting the CCME requirements for establishing a Type A water quality guideline using a Species Sensitivity Distribution (SSD). Data for the relationship between water hardness and chronic toxicity of nitrate for four species were used to produce a pooled relationship between water hardness and toxicity, and this relationship was used to normalize the

data to a single hardness. The SSD of the hardness-normalized toxicity data resulted in an HC_5 of 6.5 $mg \cdot L^{-1}$ NO_3-N at a hardness of 60 $mg \cdot L^{-1}$, and the hardness relationship resulted in SSWQOs that ranged up to 16.4 $mg \cdot L^{-1}$ at a hardness of 160 $mg \cdot L^{-1}$. The SSWQO was compared with results from toxicity tests conducted across a range of hardness values, all of which fell above the proposed SSWQO, indicating that the objective is conservatively protective across a range of water hardnesses. Further site-specific tests were conducted to increase the confidence in the safety of the SSWQO. Tests were conducted using nitrate added into waters that were characteristic of the lakes at the site using an invertebrate (*Ceriodaphnia dubia*) and two fish (*Pimephales promelas* and *Salvelinus namaycush*). An additional reduction in toxicity of nitrate was evident in the site waters, over-and-above that established on the basis of hardness, providing an additional level of safety associated with the SSWQO.

In vitro immunotoxicology of quantum dots and comparison with dissolved cadmium (Cd) and tellurium (Te) (PL)

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The increasing use of products derived from nanotechnology has raised concerns about their potential toxicity especially at the immunocompetence level in organisms. This study compared the immunotoxicity of CdS/CdTe quantum dots (QDs) and their dissolved components, $CdCl_2/TeO_2$ salts and a $CdCl_2/TeO_3$ mixture, on four animal models: one bivalve (*Mytilus edulis*) one fish (*Oncorhynchus mykiss*) and two mammals (mice and humans). Ours results of viability and phagocytosis biomarkers revealed that QDs were more toxic than dissolved metals for blue mussel. For the other species, dissolved metals (Cd, Te and Cd/Te mixture) were more toxic than the nanoparticles. The most sensitive species towards QDs was human (EC_{50} = 217 $\mu g \cdot mL^{-1}$). Lymphoblastic transformation in mouse was decreased for small QDs concentrations (EC_{50} = 4 $\mu g \cdot mL^{-1}$) and was more sensitive than other models species tested. Discriminant function analysis revealed that blue mussel hemocytes were able to discriminate the toxicity of QDs, Cd, Te and Cd-Te mix (Partial Wilk's lambda = 0.021 and $p < 0.0001$). For rainbow trout, the immunotoxic effects of QDs were similar to those obtained with the dissolved fraction of Cd and Te mixture. For mouse, the toxicity of QDs markedly differed from those observed with Cd, Te and dissolved Cd-Te mixture. For human cells, the immunotoxic effects of the QDs were similar to those observed with Cd, Te and Cd/Te mix. The results also suggest that aquatic species responded differently to these compounds than vertebrates, which led to the recommendation that mussels and mouse were most able to discriminate the effects of Cd-based nanoparticles from the effects of dissolved Cd and Te at the immunocompetence level.

The effect of chronic exposure to produced water on growth and food consumption of juvenile Atlantic cod (*Gadus morhua)* (PO)

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The discharge of produced water (PW) from offshore oil and gas facilities remains an environmental concern due to uncertainty regarding its fate, transport and potential biological effects. Rapid dilution of the oil-related discharge containing poly-aromatic hydrocarbons, phenols, alkylphenols and heavy metals occurs. Marine fish may be chronically exposed to diluted PW at distances far from oil and gas production sites. In this study juvenile Atlantic cod were exposed intermittently to 0, 100 or 200 parts per million (ppm) of PW from either Sea Rose floating production, storage and offloading vessel (FPSO), Thebaud or Venture platforms for 14 weeks. Sea Rose is an oil production facility while Thebaud and Venture are gas production facilities. Three times weekly the fish were fed and then exposed to PW. Growth and food consumption were monitored. At the end of the exposure, significant effects on specific growth rate (SGR) were seen in fish exposed to 200 ppm Sea Rose PW ($p < 0.005$). Effects on daily food consumption (FC) were significant ($p < 0.005$) for all but one group of treated fish at the end of the exposure. The results suggest that chronic exposure to environmentally relevant concentrations of PW from both oil and gas platforms may pose a risk.

Does holding back juvenile trout growth affect copper toxicity tolerance? (PO)

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Toxicity testing is a common method of practice when trying to establish guidelines and regulations for acceptable limits of chemicals being received in the environment. The aim of this research was to determine if some of the variability in LC_{50} values found in the literature for the same toxicants is due to researchers and/or hatcheries maintaining (zero growth) fish for extended periods of time prior to exposure. A standard 24-hour flow-through LC_{50} of 44.5 $\mu g \cdot L^{-1}$ (± 3.54) was determined for larval rainbow trout (~1.8 g wet weight) continuously exposed to copper. Another 24-hour continuous flow-through LC_{50} of 50.4 $\mu g \cdot L^{-1}$ (± 3.98) was determined after 21 days of holding at restricted rations (0.4% body weight/day), the rainbow trout were approximately 2.1 g wet weight. There was no significant difference seen between these two LC_{50} values. Following another 21 days of holding at restricted rations, fish were ~2.8 g and a 24-hour continuous flow-through LC_{50} of 66.5 $\mu g \cdot L^{-1}$ (±1.41) was calculated.

This final LC_{50} value was significantly different ($p \leq 0.05$) compared to the first and second values. The significant difference in the final LC_{50} value was likely due to the modest growth of the juvenile trout. Based on these findings, it is highly unlikely that the variability in LC_{50} values in the literature is due to the holding history of stock populations, including the use of restricted rations.

Sub-lethal effects of the anti-sea lice therapeutant emamectin benzoate on clam worms *(Nereis virens)* (PO)

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The clam worm, *Nereis virens*, is being considered as an organic extractive species to be co-cultured with salmon in integrated multi-trophic aquaculture (IMTA). Ecto-parasites, also called sea lice, often infest farm-reared salmon, and therapeutants are used to control those infestations. One drug, emamectin benzoate (EB), is applied as an in-feed additive, and small quantities of treated food as well as faeces containing EB make their way to the ocean bottom. As a result, sediments near salmon cage sites can contain concentrations of EB as high as 360 $\mu g \cdot kg^{-1}$ dry weight (dw). EB may persist for months or years and potentially affect co-cultured sediment dwellers such as *Nereis*. In order to assess the effects of EB on *Nereis* over a grow-out period, a 30-day bioassay was performed wherein worms were exposed to an environmentally-relevant concentration of EB in sand. Sand was replaced every 10 days with worms being weighed and their time to burrow being recorded at each change of substrate. While there was no significant mortality, there were marked changes in worm behaviour and weight. Unexposed worms gained weight over the 30 days while most exposed worms lost weight. Exposed worms emerged from sediment and did not re-burrow; by 16 days more than 50% of exposed worms were on the surface. Additionally, sand in beakers containing unburrowed worms became anoxic. These results indicate that environmentally-relevant concentrations of EB may affect growth and behaviour of *Nereis* and may limit the use of this species in an IMTA setting.

Dehydroabietic acid (DHAA) alters energy metabolism and the effects of 17β-estradiol in rainbow trout *(Oncorhynchus mykiss)* (PO)

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Recent research has suggested that dehydroabietic acid (DHAA), a resin acid present in pulp and paper mills, may have anti-estrogenic effects in fish. A 28-day chronic-exposure toxicity experiment using immature rainbow trout was conducted in order to assess the endocrine disrupting and liver metabolic effects of the model estrogen 17β-estradiol (E2) and the wood extractives DHAA and β-sitosterol (BS), regularly present in pulp and paper mill effluents. It was found that exposure to 5 $mg \cdot kg^{-1}$ of E2 significantly increased vitellogenin (VTG), hepatosomatic index (HSI), and plasma sorbitol dehydrogenase (SDH). The effects of E2 on HIS and SDH were reduced by mixing E2 with DHAA, indicating that DHAA does not cause its anti-estrogenic effects indirectly due to liver damage. Exposure to 0.05, 0.5 and 5 $mg \cdot kg^{-1}$ of DHAA caused significant increases in liver citrate synthase (CS) activity after 7 days, yet, the fish returned to control values by 28 days. These findings indicate that DHAA may alter energy metabolism as well as alter the overall effect of E2 in juvenile rainbow trout.

Effects of agricultural land use on the transfer of nutrients into stream food webs in Grand Falls, New Brunswick (PO)

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This research uses the transfer efficiency of nitrogen and phosphorus into stream food webs as an integrative measure of ecological change associated with agriculture. During the summers of 2010 and 2011, aqueous nutrient concentrations (throughout summer) and invertebrate and fish communities (late summer) were sampled from 14 streams (0-90% agricultural land use) in Grand Falls, New Brunswick. Community biomass estimates were combined with measures of elemental content to quantify the amount of nutrients stored within the animal tissues. Biotic nutrient storage was compared to the concentration of the respective nutrient in the stream water, and resulting ratios were used to indicate the efficiency of nutrient transfer into the food web. Aqueous nutrient concentrations were positively related to the intensity of agriculture in both years, and nitrogen was more strongly influenced than phosphorus. Nutrient storage in the animal communities was not explained by agricultural intensity, and site estimates differed between years and animal communities. Resulting ratios indicated that nitrogen transfer efficiency was typically lower in agriculture-impacted than in reference sites, but that phosphorus transfer was more variable. Sites with higher densities of salmonids had greater phosphorus—and to a lesser extent nitrogen—transfer into the fish community. In the invertebrate community, phosphorus transfer, followed by nitrogen, was related to total community biomass rather than the density of a single taxonomic group. These results indicate that nutrient transfer efficiency is reduced when agriculture has a strong effect on aqueous nutrient concentrations and is sensitive to factors influencing salmonid and invertebrate production.

Assessing location and temporal variation in the sensitivity of natural periphyton communities to, and ability to recover from, herbicide exposure (PO)

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Periphyton communities are composed of heterotrophic microorganisms, cyanobacteria, and algae that are fixed to a substrate by a polysaccharide matrix. They have been historically under-represented in effects assessment, but are increasingly being incorporated into toxicity testing for risk assessments of contaminants in aquatic systems. In this study, field-derived periphyton communities were collected at four different time points (April to August 2012) from six different sites across the Mid-

western United States to evaluate the sensitivity to, and ability to recover from, herbicide exposure. Pulse amplitude modulated (PAM) fluorometry was used to measure changes in light-adapted photosynthetic yield of the communities to an acute atrazine exposure (24 and 48 hours). Response (EC_{10}s and EC_{50}s) varied with sampling site, time of sampling, and duration of exposure. There were no trends in sensitivity over the sampling period across all six sites. Photosynthetic yield of the periphyton communities recovered to the level of controls within 24 hours of atrazine removal. Recovery was consistently observed across exposures, sampling sites, and sampling dates. Therefore, we conclude that there is a low risk that pulsed herbicide exposure causes chronic effects in periphyton communities.

For whole effluent testing, NOE means No Observable Effect (PO)

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With the recent and justified calls for the final demise of NOELs/NOECs , a non-toxic serial dilution toxicity test is characterized by stating that ECx > 100%, without reference to effect levels. However, following the implementation of routine industrial toxicity testing under Ontario's Municipal Industrial Strategy for Abatement (MISA) program, there has been little or no mortality or change in growth or reproduction in more than 75% of the whole effluent tests I have reviewed. Clients understandably ask if there is a statistically defensible means of comparing test results to the control. The ANOVA design incorporated into most toxicity analysis programs provides a ready means of assessing the effect of effluent concentration for the *Ceriodaphnia* and larval fathead minnow toxicity tests conducted in accord with Environment Canada's methods. A whole effluent toxicity test could be considered to have no observable effect if it met the following criteria: 1) The ECx was > 100%; 2) No dose response is visually apparent, with the response (survivorship, growth or reproduction) essentially flat across the concentration gradient; 3) Concentration is not a significant factor (at $p < 0.5$) in an ANOVA of effluent concentration versus the dependent variable; and 4) The observed effect at any given concentration is within limits permitted for controls in the test (e.g., mortality less than 10%). A similar approach might also be applied to Pass/Fail mortality tests in which there is replication of samples.

Toxicological applications of a yellow perch *(Perca flavescens)* cell line (PO)

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Yellow perch (*Perca flavescens*) is an economically important fish species found throughout North America, including water bodies in the Athabasca Oil Sands region. As such, *P. flavescens* has been a model organism for toxicological studies, including the evaluation of oil sands processed waters (OSPW). The wide ecological impact of OSPW has been of increasing concern, and high-throughput, rapid, inexpensive assays are needed. Aquatic toxicological *in vitro* models could be used as preliminary screening tools; and fish cell lines in combination with rapid bioassays to evaluate the toxicity of environmental contaminants, as well as to screen ameliorating compounds for remediation strategies, are gaining increasing interest. Since no yellow perch cell lines are available, we report here on the development of a Yellow Perch Fin cell line, YPF5, and its potential uses in toxicology. This is a fibroblastic cell line derived from the caudal fin of an adult yellow perch and has been maintained for over a year. The cells grow well at ambient temperatures in Leibovitz-15 media supplemented with 10% fetal bovine serum. Karyotyping (2n = 48) and "DNA barcoding" positively identified YPF5 as being derived from *P. flavescens*. Fluorometric assays using Alamar Blue, CFDA-AM, and Neutral Red were used to evaluate the toxicity of OSPW samples and model chemical contaminants such as naphthenic acids, ammonium chloride and copper sulphate. The sensitivity of YPF5 to these and other chemicals was comparable to reported literature data for whole yellow perch, thus this cell line could be very useful for aquatic toxicological studies.

Assessing the aquatic toxicity of amine and nitrosamine compounds in carbon capture systems (PO)

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Aquatic toxicity studies were conducted on proprietary amine compounds (Amine 1 and Amine 2) used in combination as a solvent in the CANSOLV Technologies Inc. CO_2 and SO_2 capture systems, as well as the associated nitrosamine degradation product (Nitroso-Amine). CANSOLV CO_2 and/or SO_2 capture systems treat flue gas streams for various applications such as coal-fired power plants, smelters, or fluid catalytic cracking units (FCCUs). Three acute bioassays – OECD 203 rainbow trout, OECD 202 daphnia, and OECD 201 algae – were conducted along with two chronic toxicity bioassays, the 7-day fathead minnow, and the 3-brood *Ceriodaphnia* reproduction. All acute toxicity studies for both amine compounds and Nitroso-Amine were found to have

LC_{50} > 100 $mg \cdot L^{-1}$ except in algae, Nitros-Amine had EC_{50} of 7.3 $mg \cdot L^{-1}$ (6.3-8.4 CI) and 3.8 $mg \cdot L^{-1}$ (3.4-4.3 CI) for growth and yield respectively. No chronic toxicity was observed to the fathead minnows and *Ceriodaphnia* for survival (LC_{50} > 100 $mg \cdot L^{-1}$). For reproduction effects in *Ceriodaphnia*, the IC_{25} was > 100 $mg \cdot L^{-1}$ for Amine 2, while Amine 1 had an IC_{25} of 72 $mg \cdot L^{-1}$ (53-89 CI) and Nitroso-Amine had a IC_{25} of 34.7 $mg \cdot L^{-1}$ (11.7-60.4 CI). These tests support the safety of the CANSOLV solvents and that they are considered to be practically non-toxic with low aquatic hazard. The degradation product Nitros-Amine, although found to be toxic to algae, is not expected to be emitted at significant concentration to cause adverse effects to aquatic organisms.

Reconstructing the past nutrient status of Lake Diefenbaker, a Canadian Great Plains reservoir, using paleolimnological techniques (PO)

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Lake Diefenbaker, a reservoir created in 1967, supplies drinking water to 65% of Saskatchewan's population. Anecdotal evidence suggests an increasing frequency of cyanobacterial blooms within the reservoir. Blooms typically emerge when nutrients, such as phosphorus, become sufficient and thus there is a need to investigate nutrient loading trends. A paleolimnological study is being conducted to reconstruct the historical nutrient status of Lake Diefenbaker using physicochemical variables and subfossil remains archived in depositional sediments. Cores of sediments were collected along the reservoir to evaluate a spatial gradient. Cores were vertically sectioned into 1-cm increments and a subset were analyzed for total phosphorus and three sedimentary forms of phosphorous (apatite inorganic, non-apatite inorganic, and organic phosphorus) to determine temporal trends in nutrient loading. Subfossil remains of diatoms were isolated from each section and taxonomically identified (minimum of 300 valves per sample). Results indicate that phosphorus loading and diatom community compositions have remained relatively constant at locations closest to the inflow from the South Saskatchewan River, which suggests that nutrient status has remained constant over time. However, increased phosphorus loading and shifts in diatom community composition were observed in cores collected from locations down reservoir, closer to Gardiner dam (the primary outflow of the reservoir), which suggests a trend towards increased nutrient status. Increases in total phosphorus were predominately due to increases in non-apatite inorganic phosphorus, likely caused by increased reservoir productivity. Future investigations of concentrations of sediment metals and stable isotope ratios will help to better understand nutrient dynamics and to trace organic matter sources within Lake Diefenbaker.

Toxicity of untreated and ozone-treated oil sands process-affected water to early life stages of the fathead minnow *(Pimephales promelas)* (PO)

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The Alberta oil sands are the world's second largest reserve of petroleum. There is growing concern regarding the environmental impacts of the increasing volumes of oil sands process-affected water (OSPW) being produced. OSPW is acutely and chronically toxic to fish and other aquatic organisms, but the mechanisms of toxicity are poorly understood. Early life stages of fathead minnows exposed to OSPW have a greater incidence of hemorrhaging, premature hatching, pericardial edema, and spinal malformations. The toxicity of OSPW is thought to be mostly attributable to water soluble organic compounds, in particular naphthenic acids (NAs). Ozonation of OSPW might be an effective method for remediation by reducing concentrations of dissolved organic compounds, including NAs. This study examined the effects of untreated and ozone- treated OSPW (O3-OSPW) on embryos of fathead minnows. To elucidate the potential mechanism(s) of toxicity, the transcript abundance of genes involved in biotransformation of xenobiotics, response to oxidative stress, and regulation of apoptosis were quantified. Embryos exposed to OSPW had significantly greater transcript abundance of CYP3A, GST, SOD, Casp9 and ApoEn by 2.35±0.34, 2.15±0.26, 3.08±0.74, 3.26±0.57 and 2.38±0.25-fold, respectively, than those exposed to control waters. This indicates that exposure to OSPW might cause oxidative stress and apoptosis, leading to the development of malformations. None of the effects observed in the embryos exposed to OSPW were observed in those exposed to O3-OSPW. The results suggest that the dissolved organic fraction of OSPW impairs development of embryos of fathead minnows through oxidative stress and apoptosis.

Oxidative damage in American lobster exposed to low concentrations of the anti-sea lice pesticide Salmosan® (PO)

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In the Bay of Fundy, New Brunswick, sea-lice outbreaks in caged salmons are treated with various pesticides including Salmosan® (a.i. azamethiphos), applied as bath treatments and then released in the sea. Non-target organisms such as the American lobster, *Homarus americanus*, living around the cages may suffer impacts resulting from chronic exposure to low concentrations of pesticides. In this study, adult lobsters were exposed continuously for 10 days in sea water (30 parts per thousand, 11-12.5°C) to environmentally realistic concentrations of Salmosan®, 125 and 250 $ng \cdot L^{-1}$, 1000-times

lower than the therapeutic dose (100 $\mu g \cdot L^{-1}$). Approximately 50% of the lobster exposed to the highest concentration died whereas no mortality was observed at the lowest concentration. Oxidative damage to lipids and proteins was assessed by measuring Thiobarbituric Acid Reactive Substances (TBARs) and protein carbonyls (PCs) in gills and hepatopancreas of surviving lobsters. PCs were elevated in the gills but not in the hepatopancreas of lobster exposed to sublethal concentrations of Salmosan®. No effect on TBARs in hepatopancreas or gills was detected. Further work will explore the physiological consequences of the observed oxidative damage and will assess the potential use of gill protein carbonyls as a biomarker to monitor the impacts of aquaculture therapeutants in lobsters captured close to salmon aquaculture sites.

A gonadal explant assay to assess the sensitivity of three North American fish species to disruptors of steroidogenesis (PO)

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There is concern regarding the exposure of aquatic organisms to chemicals that can interfere with the endocrine system. One critical mechanism of endocrine disruption is impairment of steroidogenesis, which can lead to altered hormone levels, altered or delayed sexual development, and ultimately reproductive failure. There is also a need to address the large number of animals required by current toxicity testing approaches, particularly when working with species native to environments of concern. The aim of this study was to develop an *in vitro* gonadal explant assay that enables the assessment of effects of endocrine disrupting chemicals (EDCs) on sex-steroid production in wild fish species. Female northern pike (*Esox lucius*), walleye (*Sander vitreus*), and white sucker (*Catostomus commeroni*) were sampled from Lake Diefenbaker, Saskatchewan, at pre-spawn and post-spawn time points. Gonads were excised and exposed for 24 hours to a model inducer (forskolin) and inhibitor (prochloraz) of steroidogenesis. Hormone concentrations in media were quantified using HPLC-MS/MS. Exposure of pre-spawn tissues to forskolin showed a concentration dependant increase in testosterone, which was significantly lesser than that in post-spawn tissue. Exposure of post-spawn tissues to prochloraz resulted in a dose dependant increase in testosterone and decrease in estradiol, with white sucker and walleye having the greatest sensitivity to prochloraz and forskolin, respectively. This study suggests that this *in vitro* method represents a useful tool in assessing species-specific responses to disruptors of steroidogenesis, with the time point of exposure being of great importance.

How to reduce ammonia toxicity as a confounding factor in marine sediment bioassays (PO)

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[1]*Maxxam Analytics*

Ammonia often occurs at acute and/or sublethal toxic concentrations to organisms in marine sediments rich in fine particles and organic matter. When assessing toxicity in a standard bioassay test, total ammonia nitrogen (TAN) can be a confounding factor often masking the effects of other contaminants of concern (COC) in sediments. Sediments collected for dredging purposes often have a high TAN toxic potential due to the metabolism of micro-organisms and anaerobic conditions in the sediment. Marine sediment samples assessed for harbour or waterway management in Québec with the marine polychaete *Polydora cornuta* showed unexpectedly high levels of toxicity compared to the level of COC present. *P. cornuta* is sensitive to many contaminants of concern, but is also very sensitive to TAN (porewater LC_{50} = 21.7 mg TAN·L^{-1} and growth inhibition porewater IC_{25} <9.4 mg TAN·L^{-1}). Treatments with Zeolites and U. lactuca can be used in Toxicity Reduction Evaluations of sediments to identify or mitigate low to moderate levels of ammonia toxicity (EPA/600/R-07/080). However, they may not be effective in mitigating the effects of very high TAN concentrations in dredged sediments. Decision makers (regulators and consultants) would like to be able to observe the potential effects of COC in sediments without the confounding effects of acute ammonia toxicity. Maxxam has proposed a decision tree and a modified protocol for *P. cornuta* in order to work with elevated TAN levels in sediments and thereby avoid the confounding effects of ammonia in sediment bioassays.

Toxicity of fluoride to a variety of aquatic species and evaluation of toxicity modifying factors (PO)

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[1]*Nautilus Environmental,* [2]*Cameco Corporation*

There is relatively little information available on the toxicity of fluoride to aquatic species, particularly for chronic exposures. As a result, the current British Columbia water quality guideline for fluoride is based on acute toxicity data for trout and incorporates a relatively large safety factor to accommodate the uncertainty associated with the small data set. The purpose of this study is to expand the data set of acute and chronic toxicity of fluoride to a variety of aquatic species. In addition, toxicity modifying factors for fluoride were also evaluated. Acute toxicity exposures were conducted on *Hyalella azteca* and *Oncorhynchus mykiss*. Chronic toxicity exposures were conducted on *H. azteca, Chironomus dilutus, Ceriodaphnia dubia, O. mykiss, Pimephales promelas, Lemna minor* and *Pseudokirchneriella subcapitata*. The most sensitive species tested was *H. azteca*. Hardness, alkalinity and chloride were

investigated as potential toxicity modifying factors of fluoride. The results from this study suggest that chloride plays a major role in modifying toxicity for the majority of species tested.

Sensitivity of *Hyalella azteca* to strontium, vanadium and potassium (PO)

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[1]*Nautilus Environmental,* [2]*Rescan Environmental Services,* [3]*Golder Associates*

The available data set for chronic toxicity of strontium, vanadium, and potassium is relatively small and water quality guidelines have not been developed for these elements. Consequently, there is a need for a better understanding of toxicity thresholds for these materials. Tests were conducted to address this data gap using the freshwater amphipod *Hyalella azteca*. The results are discussed in the context of relative sensitivity compared with other published data for other species, as well as the ratios of results from acute and chronic tests. The relative toxicity of these three materials is vanadium > strontium > potassium.

Gasoline constituent toxicity in a freshwater system: A case study using a *Ceriodaphnia dubia* laboratory test (PO)

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The use of laboratory toxicity tests to evaluate mixtures of volatile contaminants in field-collected samples is inherently problematic. Contaminant concentrations vary spatially and temporally, and proportionally within mixtures. Volatilization/degradation during toxicity testing makes identifying concentrations associated with effects to organisms challenging.

SLR implemented a toxicity testing program of gasoline-contaminated groundwater adjacent to a salmon-bearing stream impacted by a tanker spill in British Columbia. A chronic, static renewal, *Ceriodaphnia dubia* survival and reproduction test was considered the most practicable to assess potential toxicity to freshwater organisms. The six-day test, conducted by Nautilus Environmental, quantified potential effects of the hydrocarbon mixture to aquatic organisms.

The results of a 48-hour pilot test were used to determine the range of groundwater dilutions for the chronic test. The LC_{50} and IC_{20} (reproduction) sample concentrations (% volume/volume) were identified from the test results. Exposure concentrations of BETX and VHw6-10 (C6-10 hydrocarbons) were estimated using sub-sample results from three of seven dilutions collected at 24-hour intervals. The sub-

samples, collected immediately before and after daily test water renewal, allowed contaminant losses to be quantified. Mean exposure concentrations were estimated for all dilutions based on the sub-sample results.

The estimated test concentrations, supported by the results of a literature review, allowed toluene, xylene, and VHw6-10 to be identified as the primary contributors to the observed adverse effects to *C. dubia*. The estimated toluene and xylene concentrations that resulted in adverse effects to test organisms were lower than *C. dubia* effects data from previous laboratory studies using the individual substances.

The dose not the concentration makes the poison: Aquatic toxicity under changing water concentrations (PO)

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Dynamic exposures are the norm for field exposures and studies attempting to simulate field conditions. Using theoretical and case studies, we review dynamic contaminant exposures to aquatic organisms to investigate variables important for interpreting toxicity. Because the magnitude and timing of peak absorbed concentration change with exposure dynamics, interpreting the toxic response is generally limited to the specific experiment. Extrapolation to other experiments with different exposure dynamics, or to the field where exposure dynamics can vary, requires substantial information about toxicokinetics and exposure dynamics along with temporal threshold data for extrapolation between studies. This is particularly true for mixture exposures, where the concentration and composition and, therefore, the timing and magnitude of exposure to individual components of different potency can vary. For the short term, initial water concentration can be considered a conservative measure of exposure, although the extent of conservatism will vary with the dynamics of exposure and the toxicokinetics of the chemicals of interest. A better metric for interpreting toxicity is the peak absorbed dose, although this neglects toxicodynamics and requires temporal measures of accumulated dose so that the peak concentration can be determined. Aquatic toxicology studies are needed that develop temporal thresholds for absorbed toxicant doses. Such studies would allow for better extrapolation between conditions of dynamic exposure. Improved experimental designs are also needed, including high quality temporal measures of both the exposure and the absorbed dose.

The effect of pH and dissolved organic carbon on the acute toxicity of zinc to *Hyalella azteca* (PO)

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The impact of pH and dissolved organic carbon (DOC) on the acute (7-day) waterborne toxicity of zinc (Zn) to the freshwater amphipod *Hyalella azteca* was examined in tests with 2-9 day old animals in 400 mL synthetic soft water with mortality as the endpoint. Water chemistry parameters were adjusted to pH 6.5, 7.7, and 8.3 and dissolved organic carbon (DOC) concentrations of 2, 5, and 10 mg $C \cdot L^{-1}$. Based on nominal Zn water concentrations, the LC_{50} of Zn to *H. azteca* had a U-shaped response with increasing pH. The highest mortality was observed at pH 7.7. The lower toxicity at pH 6.5 could be due to increased competition for binding sites with H^+ ions on the biotic ligand, while the lower toxicity at pH 8.3 could be due to increased complexation with anions such as OH^- and CO_3^-. Higher DOC concentrations reduced the toxicity of Zn to *H. azteca*. Future work includes the determination of the impact of variable alkalinity on the acute toxicity of Zn to *H. azteca*, as well as determination of the effect of water chemistry on the chronic (4 week) bioaccumulation and toxicity of Zn to *H. azteca*.

ATW membership survey says... (PO)

CRAIG, G. [1] ***and BURRIDGE, L.*** [2]

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The Board of Directors of the Aquatic Toxicity Workshop (ATW) canvassed the membership in March and April of 2012 to gauge their sentiment about the location of future workshops, themes and topics, costs, motivation for attending, and the inclusion of Sunday ancillary events and workshops. Over 230 members responded by answering graded scale questions and providing written comments and suggestions. While the venue is not critical to attendance, the overall cost and ease of travel is influential in selecting ATW among the host of other conferences. Receiving approval to attend among government employees is a major determinant and the general economy affects the ability of private sector individuals to attend. The principle motivation for those attending is to network with peers personally and share advances in their craft at a national level. It is the reporting of recent findings and review of past initiatives that keeps the annual program fresh. Join the ATW Directors at our poster throughout the workshop to review the response graphs and read the numerous comments left by respondents. Leave your own ideas on how future workshops should be directed.

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